Universidade de Aveiro Departamento de Biologia
Ano 2017

BIANCA RAQUEL CORREIA DOS REIS

CONSEQUENCES OF MIGRATORY CHOICES AND MERCURY ACCUMULATION IN CORY'S SHEARWATERS

CONSEQUÊNCIAS DAS ESCOLHAS MIGRATÓRIAS E DA ACUMULAÇÃO DE MERCÚRIO NAS CAGARRAS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha, realizada sob a orientação científica da Prof. Doutora Eduarda Pereira do Departamento de Química da Universidade de Aveiro e do Prof. Doutor Paulo Catry, MARE – Marine and Environmental Sciences Centre, ISPA – Instituto Universitário.

DECLARAÇÃO

Este relatório é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

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palavras-chave

Locais de invernada, mercúrio, efeitos carry-over, reprodução, data de chegada, condição corporal

Resumo

As escolhas migratórias das Cagarras (*Calonectris borealis*) têm um potencial impacto nos indivíduos, podendo produzir efeitos “carry-over” com implicações na estrutura e dinâmica populacional.

Os principais objetivos deste estudo foram: comparar a distribuição de uma população estudada de cagarras da Selvagem Grande durante o período não reprodutor; investigar se o local de invernada teve algum tipo de influência na data de retorno à colónia, na duração da migração, condição corporal e sucesso reprodutor; observar como estes fatores se relacionam com a acumulação de mercúrio (Hg) nos indivíduos no local de invernada; e averiguar a distribuição deste contaminante na espécie em estudo.

A maioria dos indivíduos migrou para regiões oceânicas em torno do sul de África. Os indivíduos que invernaram na Corrente das Canárias eram todos machos e os primeiros a retornar à colónia na Selvagem Grande. Relativamente ao sucesso reprodutivo, o sucesso na colocação e eclosão do ovo foi elevado e semelhante a valores registados em estudos anteriores para outras aves marinhas. As fêmeas amostradas em março tiveram uma maior percentagem de ovo no ninho do que os machos. O sucesso na colocação de um ovo não aumentou com a antecedência na chegada. Os machos da corrente das Canárias tiveram uma taxa de sucesso de colocação de ovo de 57.9% e os da região sul Africana 75.4%. Não se verificou qualquer relação entre condição corporal e reprodução e entre condição corporal e data de chegada, apesar de os machos da corrente das Canárias terem registado valores um pouco mais reduzidos. A concentração média de mercúrio acumulada em penas que cresceram durante o período de invernada das cagarras foi de 5,8±2,3 mg/kg, sem se terem registado diferenças entre machos e fêmeas. Os machos que invernaram na corrente das Canárias registaram os níveis mais baixos de mercúrio comparando com os da região sul Africana. As concentrações de mercúrio derivadas da exposição nos locais de invernada não afetaram os níveis de sucesso na colocação de ovo, nem a duração da migração, nem a condição corporal entre os indivíduos. No entanto, registou-se uma fraca correlação positiva entre datas de chegada dos machos da região sul Africana e concentrações de mercúrio, na qual os indivíduos que chegaram mais tarde tinham níveis mais baixos de mercúrio. Foi, portanto, possível observar um certo nível de influência das escolhas migratórias e também efeitos “carry-over” quer ao nível da escolha do local de invernada, quer ao nível de concentrações de mercúrio nestes indivíduos.

Os dados obtidos fornecem informação acerca da distribuição da espécie e influencia das suas escolhas migratórias, o que pode ser usado para medidas de conservação. A utilização da espécie como biomonitor de níveis de mercúrio é também um meio de obter informação sobre os níveis deste contaminante no ambiente aquático, terrestre e na atmosfera.

Keywords Wintering grounds, mercury, carry-over effects, reproduction, arrival time, body condition

Abstract

The migratory decisions of Cory's shearwaters (*Calonectris borealis*) potentially have an impact on individuals, producing carry-over effects, with implications for population structure and dynamics.

The main goals in this study were to compare the distribution of a studied population of Cory's Shearwaters, at Selvagem Grande, during the non-breeding period; investigate the influence of wintering ground on the date of arrival at the colony and body condition on arrival and how these factors could relate with reproductive success and see how these previously mentioned factors were influenced by mercury (Hg) accumulation in the wintering ground and how this contaminant was distributed amongst wintering locations.

Most of the studied individuals migrated to oceanic regions around southern Africa. Individuals wintering in the Canary Current were all males and the first to return to breeding grounds. Relative to reproduction, overall laying and hatching success were high and close to values recorded for other seabirds. Females handled in March had a higher percentage of egg presence in the respective nest when compared to males. Laying success did not increase with early arrival dates. Males having wintered in the Canary Current had a laying success of 57.9% and those wintering in southern Africa 75.4%. Body condition did not have any relationship with reproduction and arrival date, but it seemed slightly lower for male individuals wintering in Canary Current, in comparison with the other males.

The mean mercury concentration deposited in winter grown flight feathers of adult Cory's Shearwaters was 5.80 ± 2.3 mg/kg, with no difference between males and females. Males wintering in the Canary current registered the lowest mercury burden when compared with birds spending the winter in southern Africa locations. Mercury concentrations due to the exposure in wintering grounds did not seem to affect the duration of migration, laying success neither body condition amongst individuals. However, there was a positive correlation between arrival dates from southern Africa males and mercury concentrations, in which individuals arriving later had higher mercury concentrations. It was possible to assess a certain influence and carry-over effects from wintering locations in these individuals' traits.

The collected data provides information about this species distribution and influences of its distribution in its life choices, which can be used to conservation measures. Also, using this species as a biomonitor for environmental mercury provides important insights about its levels in wintering areas.

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CHAPTER 1 – INTRODUCTION

1.1 Migration, a brief approach

Migration is one of the most spectacular and complex natural phenomena, attracting researchers, philosophers and enthusiasts for centuries. It manifests in different animal groups and is a key trait in life-history strategies (Zimmerman, 1998; Dias *et al*, 2011). True migration has regularity in time/season and location; the movements are between two distinct areas, usually one to reproduce and other to spend the winter (Webster *et al.*, 2002). Compared with other kind of movements, such as dispersal or daily foraging trips, migration usually involves a longer journey over tens, hundreds or thousands of kilometres and in much more restricted and fixed directions (Newton, 2008). While many species of fish, mammals, and even insects have impressive migratory journeys, birds as a group are one of the most mobile creatures on Earth (Zimmerman, 1998). They have adapted bodies, with a structure and physiology that allows life in the air. These adaptations make it possible for birds to seek out environments based on their needs at separate times of the year, originating the migration phenomenon (Zimmerman, 1998). The body adaptations make possible for birds to travel fast and economically over long distances, if necessary crossing seas, and other inhospitable areas. Besides morphological adaptations, birds have great orientation and navigational skills making it possible to remember and refind remote places they have previously visited (Newton, 2008). In response to seasonal changes in environmental conditions, more than 50 billion birds are thought to migrate every year on return journeys between breeding and non-breeding areas usually spending their annual non-breeding period at lower latitudes than their breeding period (Berthold, 2001; Berthold *et al.*, 2013; Newton, 2013). This results from the alternation of warm and cold seasons at high latitudes, or of wet and dry seasons in the tropics, originating a directional migration movement between breeding areas in higher latitudes to lower latitude sites during non-breeding season (Newton, 2008).

1.2 Migrant Seabirds - Order Procellariiforma

Some of the most impressive travellers on Earth are pelagic seabirds. They are one of the fastest long distance migrants, travelling thousands of kilometres and achieving travel speeds in the range of 400–1000 km per day (Dias *et al*., 2012). The patterns of migration are rather diverse and relate less to latitudinal temperature patterns controlling the movements of terrestrial birds. The most relevant factors affecting seabirds are the locations of suitable nesting areas and productive ocean waters (Cox, 2010).

Of the 234 species of birds of eighteen families which are largely or entirely pelagic in their distributions in the nonbreeding season, one group that is extremely adapted to life in the sea is the order Procellariiforma (Hoyo *et al.,* 1992). It includes a diverse group of species like storm-petrels, albatrosses, gadfly petrels, diving petrels, fulmars, prions and shearwaters (Warham, 1990; Onley, 2007).

Because these long distance migrant seabirds spend more than half of their lives in the ocean (Burger, 2013), they have evolved specific adaptations for the challenging conditions of their life history (Newton, 2008). Procellariids, aside from their migratory characteristics, have a wide-ranging pelagic behaviour, coming ashore only to breed. During breeding time, they mostly rely on making foraging excursions to the feeding areas in the sea (Pennycuick, 2009). The members of this group are long-lived species, with maximum ages ranging from four to six decades. Their body is extremely well adapted to long distance flights, using the wind to decrease the energy expended in wing movement. If the wind is favourable, the energy costs associated with flight can be as low as the ones during incubation (Pennycuick, 2002). The energy spent searching for food during migration is also low compared with other long distance migrants, due to the ability to fly close to the ocean's surface (Dias *et al.,*2012).

Most of procellariids breed in colonies that can reach hundreds or even thousands of individuals and have synchronously timed breeding cycles (Schreiber & Burger, 2001). Islands are the habitat most often chosen to breed (Lewis *et al.*, 2002) and that may be to avoid mammalian and other avian predators (Lack, 1968; Coulson, 2002). They have a late sexual maturation and breeding, with low annual reproductive rates (Schreiber & Burger, 2001; Nevitt, 2008). They are predominantly socially monogamous, probably as a response to the need for biparental care. Most of the times couples remain faithful between years, although divorces are not excluded. One egg is laid per year and parents alternate incubation and, later provisioning duties (Ricklefs, 1990; Schreiber & Burger, 2002).

1.3 Importance of studies in migration and "carry-over" effects from wintering sites

Human population and resource consumption are growing at a very high rate (Cox, 2010). The alterations made by humans in marine ecosystems are more difficult to assess and quantify than in terrestrial environments, but it is thought that they are substantial (Vitousek *et al.,*1997). In recent studies, it is shown that humans use about 8% of the primary productions of the ocean and that fraction grows to more than 25% in upwelling zones (Vitousek *et al.*, 1997; Rappole, 2013).

One of the problems related to populations levels of seabirds is that many of their life history traits that make them well adapted to the environment make them particularly susceptible to population decline and extinction (Cox, 2010; Rappole, 2013). The threat to these species not only is a great concern in terms of biodiversity but also puts at risk the migration phenomenon (Wilcove & Wikelski, 2008; Dias *et al.*, 2011). This makes it necessary to study the populations and their behaviours, as well as the environment surrounding (Furness, 1993; Furness, 2012).

Recent technological advancements have been improving our knowledge about the movements of pelagic seabirds by remote tracking, but most efforts have been focused on the breeding season (for example Catry *et al.,*2004, Hamer *et al.,*2007, Paiva *et al.,*2010a). Until recent times, conservation efforts towards these species were based on the protection of their colonies, due to the threats they suffer in terrestrial land (Croxall *et al.,*2012; Ramírez *et al.,*2013), but as the knowledge about this group and how the migrant life cycle works increases it becomes apparent that applying measures directed to just a portion of their life cycle will not necessarily act sufficiently as a conservation measure. The term "migrant connectivity", which refers to the geographical connection of populations between wintering and breeding grounds, and its study is an attempt to deal with that issue (Rappole, 2013). When a species spends its breeding season and wintering season in geographically separate places, the starting place for preservation is knowing where those environments are located, which are the major threats and where population limitation is occurring. (Rappole *et al.*, 2007; Rappole, 2013). Because of the inherent difficulty in following seabird movements and behaviour outside the breeding season little is known about the wintering period compared with the breeding season (Burger, 2013) and there are still relevant gaps about the way these birds use and interact with the marine environment (Catry *et al.,* 2004). Wintering areas, migration corridors, and population mix in winter quarters of most pelagic marine predators are unknown (González-Solís *et al.*, 2007). Nowadays, with recent technology it is possible to track those individuals, allowing the delimitation of marine important conservation areas (Bridge *et al.*, 2011).

A major factor that can interfere with procellariids populations' dynamics is that individuals may carry with them impacts of events or processes that occurred in habitats far in time and space (Harrison *et al.,*2011). Conditions experienced in distant wintering areas, for example, may have a profound influence on geographical distribution, arrival date to the breeding colony, body condition and reproductive performance at the breeding grounds, producing carry-over effects (Marra *et al.,*1998; Catry *et al.*, 2011), with implications for population structure and dynamics (Norris & 8 Taylor, 2006; Studds *et al.*, 2008; Dias *et al*., 2011). This events and processes can conduce to carry-over effects that can influence individual performance in a subsequent season (Harrisson *et al*., 2011; Catry *et al.*, 2013). However, their occurrence in the annual cycle of migratory avian taxa is scarcely studied (Catry *et al.*, 2013).

Wintering grounds' carry over effects may have to do with natural environmental characteristics from these locations, for example productivity levels, but also anthropogenic ones, like pollution and contamination of the oceans (Wilcove *et al.*, 1998; Cox, 2010; Croxall, 2012). One contaminant that can have adverse effects in birds is mercury (Seewagen, 2015). The growing awareness of mercury's toxicity in humans has increased concerns about mercury's adverse effects on other organisms, like seabirds. Ecosystems can be threatened by elevated environmental mercury levels, with higher trophic-level organisms considered most at risk. This includes pelagic seabirds, which can have high body burdens of mercury because of the biomagnification of mercury in aquatic food chains (Scheuhammer *et al.*, 2007). Some of the adverse effects associated with these elevated mercury concentrations can be reduced reproductive success (Scheuhammer *et al.*, 2015), altered behavioural traits (Scheuhammer *et al.*, 2007; Seewagen, 2010) and immunotoxicity (Scheuhammer *et al.*, 2007; Scheuhammer *et al.*, 2015). Mercury is a naturally occurring element and is released to the environment by a variety of natural and anthropogenic processes. It is then distributed and redistributed by various processes, including atmospheric exchange with both terrestrial and oceanic compartments, export from terrestrial and freshwater systems to the ocean, and deposition and burial in sediments (Sunderland & Mason, 2007). Birds are considered good bioindicators of environmental mercury contamination (Evers *et al.,* 2008) and sometimes serve in part as the basis for drafting mercury regulation policies or provide measures of success following policy implementation (Driscoll *et al.*, 2007). As such, mercury levels in birds have been widely recorded around the world (Burger *et al.*, 1992, 1993, 1997; Burger and Gochfeld, 1993; Watanuki *et al.,* 2015; Becker *et al*., 2016; Bustamante *et al*., 2016). However, relatively few studies have investigated the actual consequences of observed mercury levels on free-living birds' condition, fitness, or survival. Fewer still have examined species outside North America and Europe (Seewagen, 2010; Scheuhammer; 2015).

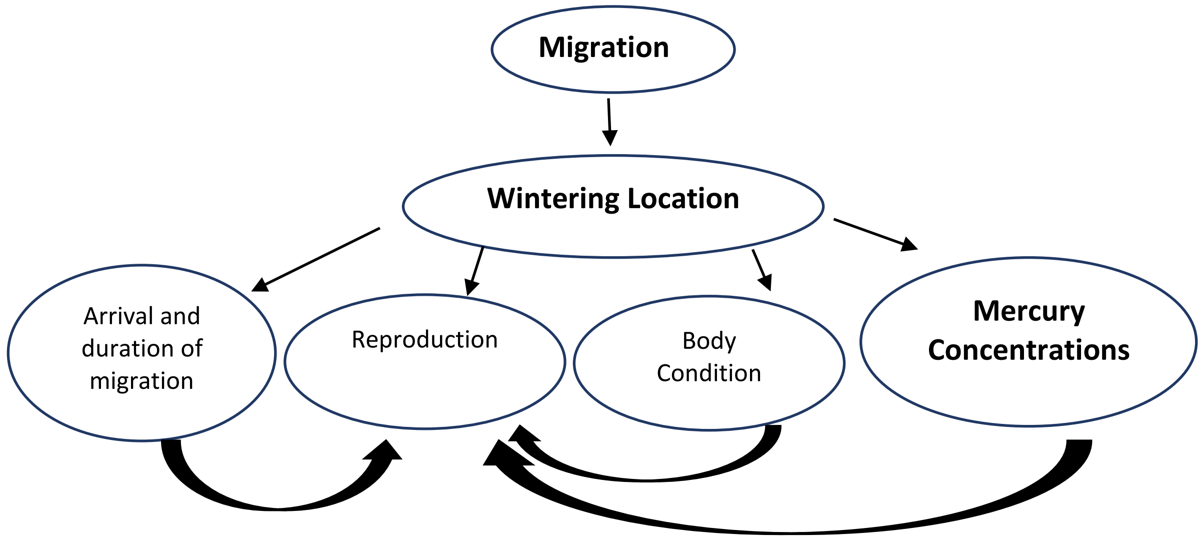
The management and conservation of marine pelagic birds most of all depends in the comprehension of this spatio-temporal dynamic between different wintering grounds characteristics and population's dynamics (Ramos & González-Solís, 2012; Ramírez *et al.*, 2013) and what can be creating "carry-over" effects in individuals traits and life cycle. In addition, a consistent knowledge and monitorization on the population trends is also necessary (Rappole, 2013).

1.4 Main goals and structure of the study

In this study the main goals were to i) compare the distribution of a studied population of Cory's Shearwaters, at Selvagem Grande, during the non-breeding period; ii) investigate the influence of wintering ground choice on the date of arrival at the colony and body condition on arrival and how these factors could relate with reproductive success and iii) relate the previously mentioned factors with mercury accumulation in different wintering grounds and see how this contaminant influenced individuals and iv) how these contaminant was distributed amongst wintering locations.

This dissertation is divided in two main parts (according with the goals described above), and these integrate different chapters (3rd and 4th chapters). In the 2nd chapter are shown the tracking effort results, allowing to see which wintering locations were chosen by Cory's shearwaters' individuals. Although the wintering locations chosen by this species are already described by literature, tracking results were needed to integrate in the main purposes of this study. In chapter 3 are described the results related to "carry-over effects" from the different wintering locations on the individuals. In the 4th chapter "carry-over" effects are also investigated but this chapter includes all the mercury analysis, as well as the monitoring component of this study.

However, it is important to note that wintering locations and mercury levels are not independent of each other and are connected in explaining the studied variables (arrival time at the colony, duration of migration, body condition and reproduction).


Migration
Wintering Location
Arrival and duration of migration
Reproduction
Body Condition
Mercury Concentrations

CHAPTER 2 – TRACKING WINTER MOVEMENTS OF CORY'S SHEARWATERS

2.1 Introduction to bird tracking

One of the most recent challenges faced by migrant seabirds researchers is to obtain knowledge about the period of time between breeding events (Ristow *et al.*, 2000). Nowadays, advances in remote tracking of individuals provide insights into their locomotion, behaviour, migration, demographics and exposure to anthropogenic risks at sea (Burger & Shaffer, 2008; Dias *et al.*, 2012). It is now possible to assess how the individuals interact with the marine environment and how it influences their distribution (Magalhães *et al.,* 2008, Paiva *et al.,* 2010a). The continuous improvement of tracking devices is a major factor contributing to Procellariids conservation measures. It is important to understand which marine locations are used by individuals (hotspots) and designate them as Marine Protected Areas (MPA's) (Hyrenbach *et al.,* 2006; Croxall *et al.,* 2012).

With oceans being rapidly affected by human activities, the information from tagged birds is extremely relevant and individuals can be used as tools for getting oceanographic and biological information on the sea itself (Furness & Camphuysen, 1997; Burger & Shaffer, 2008). Seabirds occurrence is closely associated with specific ocean processes, like continental shelves and shelf edges, upwelling systems, and fronts. These marine regions are also the ones of most interest for oceanographers (Hunt *et al.*, 1999; Burger & Shaffer, 2008). Oceanic environment is very heterogenic in terms of chemistry and physics, influencing in how its biological resources are distributed (Weimerskirch, 2007). The organisms that have this environment as a habitat are also influenced by biotic and abiotic factors that vary in a spatial and temporal scale (Fauchald, 2009; Ramírez *et al.*, 2013). Ocean's productivity is especially high in places with specific ocean currents, upwelling, and other marine phenomenon, but in general, it decreases with latitude towards equator line (Paiva *et al.,* 2010b). Seabirds movements are highly associated with these locations (Burger & Shaffer 2008).

With the information about non-breeding periods that is now available for obtaining through bird tracking, it is possible to gather different knowledge about habitat requirements, try to predict movements at sea and try to assess "carry-over" effects, comparing conditions at different places (Marra *et al.,* 1998; Catry *et al.*, 2011).

2.2 Geolocation

One way of tracking individuals is using geolocation. The most important factor driving the development of this kind of technology is studying the movements of seabirds outside the breeding period, which have revealed some remarkable migration patterns across ocean basins (Burger & Shaffer, 2008).

Geolocation, or Global Location Sensing (GLS) uses changes in ambient light levels to estimate sunrise, sunset, longitude, latitude and consequently, day length. Geographical locations are then established using astronomical algorithms (Burger & Shaffer, 2008). A geolocator registers one or two locations per day (mean error 185– 200 km) (Phillips *et al.,* 2004; Shaffer *et al.,* 2005). Despite its spatial accuracy limitations, this kind of technology has several advantages. Devices have a very low power consumption, allowing small batteries and small tags that can record locations for several years and follow birds through the whole migration process. Some of the bigger tags have a battery that can last up to ten years. This is only possible because data are stored and not transmitted (Burger & Shaffer, 2008; Rodríguez, 2008). Geolocation loggers do not require long distance signal reception, but as a disadvantage, tagged individuals need to be recaptured to download the collected information. This is a restraint because, in its majority, application is restricted to breeding birds (Burger & Shaffer, 2008; Rodríguez, 2008). Geolocators have been deployed on procellariids with no negative effects for the individuals although in many studies it did not report the effects of device deployment, only immediate behavioural changes (Burger & Shaffer, 2008; Lislevand, 2013).

2.3 Cory's Shearwater – Study Subject

Cory's Shearwaters (*Calonectris borealis*) (figure 1) are a large, brown and white procellariid seabird with a strong, distinctive flight, gliding above the ocean with only occasional deep beats of its long, often bowed wings (Mullarney & Svensson, 2003).

This species reunite several parameters that make them a good object for this study. In addition of being long-lived species, they are long-distance trans equatorial migrants whose migratory patterns can be followed using tracking technologies, like geolocators (Phillips *et al.*, 2004; Dias *et al.*, 2011). Besides, they winter in various locations, which they can choose between (Dias *et al.*, 2011). Each wintering location may have distinct characteristics and quality, depending on oceanography and human activity, and so it can induce potentially different consequences on the individuals (Behrenfeld *et al.*, 2006). Most of the population can be found in the archipelagos of Azores and Madeira (Portugal) (more than 85%) and in the Canary Islands (Spain) (15%). Few hundred pairs also breed in Berlengas (Portugal), off the Portuguese coast. In its total, population size reaches 240,000-250,000 pairs of individuals (Granadeiro *et al.*, 2006). When breeding season is over (end of October, beginning of November) they can migrate to South Atlantic, Indian Oceans or remain in North Atlantic. In southwest Atlantic it occurs in Brazil, Uruguay and Argentina, but the majority goes to Southern Africa (Hoyo *et al.,*1992).

After migrating from wintering locations, the first individuals arrive at breeding colonies between February and March and leave at the end of October, beginning of November (Granadeiro *et al.*, 2006). Cory's Shearwaters are usually nocturnal in their activity at the colony and during the breeding season come to land after the sunset (Bannerman, 1914; Servent, 1987). Its behaviour is essentially pelagic. It feeds on squids and small pelagic fish, which are obtained mainly by surface-seizing (Hoyo *et al.,* 1992). Its pelagic movements are easily divided in frequent foraging trips around breeding areas, rapid long-distance migrations and smaller scale movements within defined wintering grounds (González-Solís *et al.,* 2007).

Most threats to this species are related to the introduction of invasive species and predators in breeding grounds, habitat loss due to urban expansion, light pollution that causes disorientation in juveniles and accidental capture in fisheries (Meirinho, 2014). Although Selvagem Grande's population is already the largest in the world, its numbers have been showing a positive trend since the 1980's due to the recovery from the massacres between 1975 and 1976 (Meirinho, 2014).

Figure 1-Adult Cory's Shearwater at Selvagem Grande in Spring of 2017

Cory's shearwater's population at Selvagem Grande, in Madeira archipelago, has almost 30,000 pairs and an estimated increase of 4.6% per year since 1980 (Granadeiro et al., 2006). This colony has a distinct behaviour comparing to the other colonies since is the only one where the individual's activity is partly diurnal, the first returns to land occur before sunset during some summer weeks (Granadeiro et al., 2006). Out of the breeding colony, Cory's shearwaters spend the boreal winter in the southern hemisphere. They leave the colony at the end of October and beginning of November and then return to Selvagem Grande at the end of February, beginning of March (Zino 1971; Zino *et al.,* 1987). The eggs are laid at the end of May/beginning of June and hatch 54 days later, on average, during the fortnight of July. The chicks have a growth period of 95 days and then leave the colony with the adults (Zino, 1971; Zino *et al.,* 1987).

There are various historical records of exploitation of Cory's Shearwaters at Selvagem Grande (e.g., Bannerman, 1963; Zino, 1985). In the beginning of the 1900s, when the island was still privately owned, in excess 20,000 chicks were collected annually for meat, oil and feathers (Zino, 1985), from a population that was speculatively estimated at 130,000-150,000 breeding pairs (Mougin *et al.*, 2000). In the late 60's, population numbers suffered from heavy exploitation of chicks. In 1971 the island was classified as Nature Reserve, but it still did not have vigilance and effective protection. As a result of that lack of protection, in 1975 and 1976, several massacres by Portuguese and Spanish fishermen made a major population collapse, leaving only 64 chicks to be found in the island in 1976 (Zino, 1985). In 1980 the population was estimated at only 7000 pairs (Mougin & Stahl, 1982). Due to the mass culling events that reduced the population speculatively estimated between 130 000-150 000 breeding pairs by 90%, carried out by Portuguese and Spanish fishermen in the 1970's, Cory's population in Selvagem Grande is still recovering. Since 1977 protection measures have been implemented and the population started recovering (Granadeiro *et al.,* 2006).

2.4 Island Geography and oceanography of the Selvagens Archipelago

Field work from the present study was carried out in Selvagem Grande (30° 09'N, 15° 52'W), the biggest island from Ilhas Selvagens archipelago, which includes Selvagem Grande, Selvagem Pequena and few islets (figure 2). It is the southernmost Portuguese territory, belonging to Madeira's autonomous region and being located southeast from Madeira and north of the Canaries in the northeast Atlantic (Granadeiro 2006; IFCN, 2017). It is a place where long-term research about Cory's Shearwaters has been carried out (Mougin *et al.*, 2000; Granadeiro *et al.,*2006). This 260ha island is from volcanic origins (Granadeiro *et al.,*2006) and it is constituted by an extended plateau (with a mean of 100m of high) surrounded by cliffs (Granadeiro *et al.,* 2006; IFCN, 2017) (figure 3). Selvagem Grande is surrounded by an oceanic environment, but is situated within 375 km of a large neritic system, the African continental shelf. Ocean circulation in this region of the Atlantic is complex. Coastal upwelling, present on the northern Portuguese (Sousa *et al.,*2008) and African (Davenport *et al.,*2002) coasts, enhances the primary production of these areas (Huntsman & Barber 1977). The island is part of the Macaronesia biogeographic region and its subtropical climate is extremely dry, with temperatures a higher than in Madeira island (IFCN, 2017).

Figure 2-Selvagens' archipelago map (IFCN, 2017)

Selvagens' archipelago was the first Portuguese area to be declared Natural Reserve in 1971 (IFCN, 2017). It is also classified as an *Important Bird Area* (IBA) (Costa *et al.,* 2003), it belongs to Rede Natura 2000 since 2001 (IFCN, 2017) and it is also classified as a "Zona de Proteção Especial" under Directiva Aves and "Zona Especial de Conservação" under Directiva Habitats. All the area covered by the archipelago is a full protection area ("Área de Proteção Total") (IFCN, 2017). This habitat is classified as a comum interest habitat ("Habitat de Interesse Comunitário") (IFCN, 2017). The study site is considered a breeding spot of greater importance for marine birds of the Macaronesia and North Atlantic. Besides Cory Shearwaters other species are known to nest in this place: Bulwer's Petrel (*Bulweria bulwerii)*, white-faced storm-petrel *(Pelagodroma marina hypoleuca)*, band-rumped storm petrel (*Oceanodroma castro)*, Barolo shearwater (*Puffinus assimilis baroli)* and Yellow-legged gull (*Larus michahellis*) (Campos & Granadeiro 1999; IFCN, 2017). There are no native mammal species, with invasive rabbits and mice also being recently irradicated (IFCN, 2017).

Figure 3-Selvagem Grande's Landscape during field work in spring of 2017

2.5 *Cory's Shearwater's Wintering Locations*

Observations made at sea registered that Cory's individuals appear in various parts of the Atlantic and it suggests that most of them spend winter in the Southern Hemisphere, some penetrating the Indian Ocean (Ristow *et al.*, 2000).

Studies about Cory's Shearwater individuals from Selvagem Grande colony, such as Dias *et al.* (2011), indicate that they have six possible wintering destinations, from which they can choose: Benguela Current, Agulhas Current, central South Atlantic, Brazilian Current, Northwest Atlantic and Canary Current (Dias *et al.*, 2011) as it is shown in figure 4.

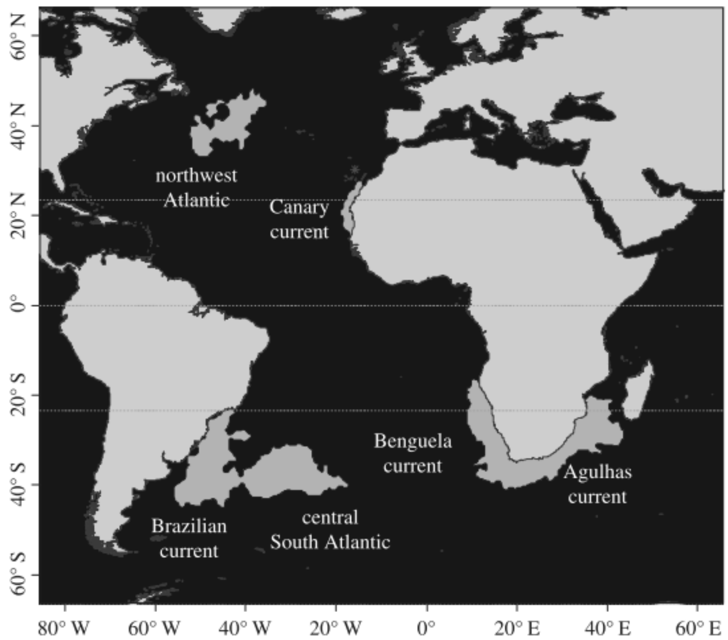


Figure 4-Cory's Shearwaters' known wintering destinations described in previous studies (Dias *et al.*, 2011)

Cory's Shearwaters' wintering locations are associated with productive areas and upwelling systems (González-Solís *et al.*, 2007; Dias *et al.*, 2012), especially in the Benguela Current Upwelling System and the Canary Current Upwelling System. These two upwelling locations represent productive regions and relatively high primary production levels throughout the year (Lachkar & Gruber, 2012). Those levels of productivity tend to vary seasonally (Kämpf & Chapman, 2016). In general, summer and spring periods register higher productivity rates, due to stronger favourable winds and higher temperatures. Therefore, the Canary Current Upwelling System is more productive during Cory's shearwater breeding season, while the Benguela Upwelling System offers more productivity during the non-breeding season (Kämpf & Chapman, 2016), suggesting that Benguela may be the most favourable wintering location when comparing with the Canary Current. According to productivity estimates, Agulhas Current seems to be a less productive region (Demarq *et al.*, 2010), suggesting that this is a less favourable wintering location when compared with Canary Current and Benguela Current.

A phenomenon that is common in many bird species and can influence population dynamics is differential distance migration of sexes and it applies in Cory's Shearwaters. It consists in individuals from each sex migrating to different places (Catry *et al.*, 2005; Pérez *et al.*, 2014). Differential distance migration of sexes could be explained by the body-size hypothesis, that suggests that winter segregation varies accordingly to individuals' size. Locations with milder climate should be home to smaller and more fragile individuals and harsher locations to larger ones (Catry *et al.*, 2005). It can also be explained by the dominance hypothesis, in which larger individuals or dominant individuals, respectively, remain closer to the breeding areas (Catry *et al.*, 2005; Pérez, 2014). Another hypothesis to explain this phenomenon is that individuals of the territory defending sex arriving early to the colony would be at advantage in terms of securing nests, avoiding confrontations (Catry, 2005).

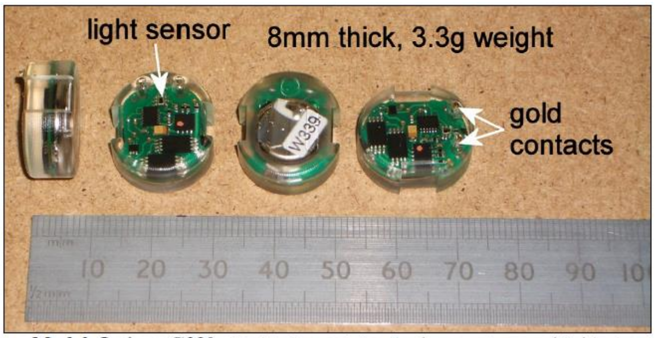
Carry over effects from the previous breeding season are known to induce partial migration. In a study done by Catry *et al.* (2013), individuals that failed breeding were found to remain resident during the following winter period and arrived earlier at the colony in the next breeding season. The fact that some individuals remain resident in Canary Current is a reproductive investment, since birds face harsher weather conditions during winter in order to arrive earlier to the colony and secure a nest (Catry et *al.,* 2013).

2.6 Tracking Effort of 2016/2017

During 2016's breeding season, 180 birds were tagged with Migrate Tech geolocators. Most of these birds formed part of the population belonging to the long term monitoring effort of the species. As a result, they are known to come back to the same group of marked nests from one year to the next and the majority of the individuals had been previously sexed.

Geolocators were attached with a leg-mounted plastic cable tie to the aluminium ring. In order to retrieve these GLS to obtain tracks of Cory's Shearwater's migration patterns and assess wintering locations field work was carried out in Selvagem Grande island (30° 09'N, 15° 52'W) between 2 of March and 12 of April of 2017. From the 180 birds tagged with geolocators, 152 were retrieved in spring 2017 with the respective geolocator tag. In order to retrieve as many geolocators as possible, nests on the island were checked on a daily basis, to amplify the chance of finding birds that just arrived. When a target bird was found in the nest, it was carefully taken out. The geolocator was removed from the bird ring, stayed ground-truthing during a period of at least 3 days and then the tracking data was downloaded with Intigeo© software and later analysed with Intiproc© software.

Geolocation loggers used in this study were Intigeo-C330 model with 17x19x8mm, 3.3g of weight (figure 5) and battery with 5 years of duration from Migrate Technology© (Figure 5). These geolocators represent between 0.9 to 1.5% of the weight from an adult individual (800g on average), which is well under the maximum recommended value 3% (Phillips *et al.,* 2003). Light data was sampled every minute with maximum light recorded every 5 minutes and they obtained two positions per day.



Model: Intigeo-C330, 17x19x8mm, 3.3g, 5yr battery (e.g. seabird leg)

Figure 5-Chosen geolocator model Intigeo-C330 by Migrate Techonology© (Migrate Techonology©, 2017)

Geolocators measure light intensity. It records light intensity in form of light curves from which sunset and sunrise times can be estimated. Latitudes are then derived on the basis of day length, and longitudes from the time of local midday and midnight. It is also relevant to have sun elevation angle at sunrise and sunset. After the positions being calculated, day and night locations are averaged to give a single location per day (Ramos *et al.*, 2012; Merkel *et al.*, 2016). Geolocation precision can be affected by various factors, including season of the year, latitude, cloudiness, artificial light sources, among others (Phillips *et al.,* 2004). In addition to these factors, two to three weeks before and after equinoxes, latitude calculations are often incorrect because day length is very similar between locations. The accuracy of latitude also decreases near the equator because of the low variation in day length (Fox, 2015). Due to that, longitude is more accurately determined than latitude for light level geolocation (Fox, 2015). Geolocation method is more accurate for areas between Tropic of Cancer and Arctic Circle and between Tropic of

Capricorn and Antarctic Circle, although adequate data can be obtained outside of these two ranges (Merkell *et al.*, 2016). Sometimes the equinox period would coincide with the return of Cory's shearwaters' individuals to the breeding colony, and in these cases the arrival date was based only in longitude values, since it is not affected by the equinox (Fox, 2015). While analysing GLS data, unrealistic positions (maybe due to interference of light curves at dawn or dusk, or around equinox periods) were dismissed from the sample (Phillips *et al.*, 2004; Fox, 2015; Dias *et al.*, 2011).

Geolocators' data were analysed using the Threshold method, which consists in predefining a light intensity threshold, used to determine day length, based on the timing of twilight events. Latitude will depend on the solar angle below the horizon at which the threshold is crossed (Dias *et al.*, 2011). Each geolocator stayed ground-truthing for 2 distinct periods of time. One before tracking and one after (before downloading), both while in Selvagem Grande. Light data were analysed in Intripoc© software in order to assess the integrity of light curves and estimate sunrise and sunset moments, which were considered to happen when the light curve intersected threshold line. The considered threshold level was 2 (Figure 6). Sun elevation angle used was linear elevation angle, calculated from calibration periods. Intiproc© generated the location maps from light data (Dias *et al.*, 2011). An example of a migratory pattern is shown in figure 7.

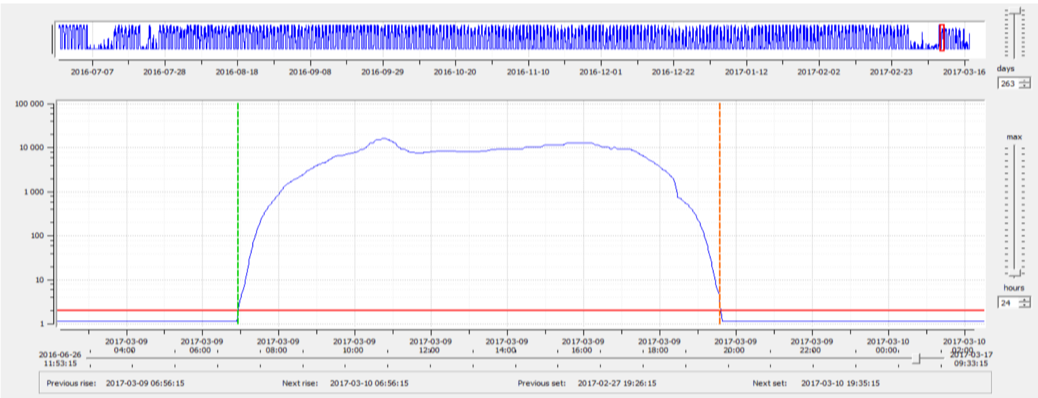


Figure 6-Light curves recorded in a geolocator with a threshold level of 2 being analized Intiproc software©

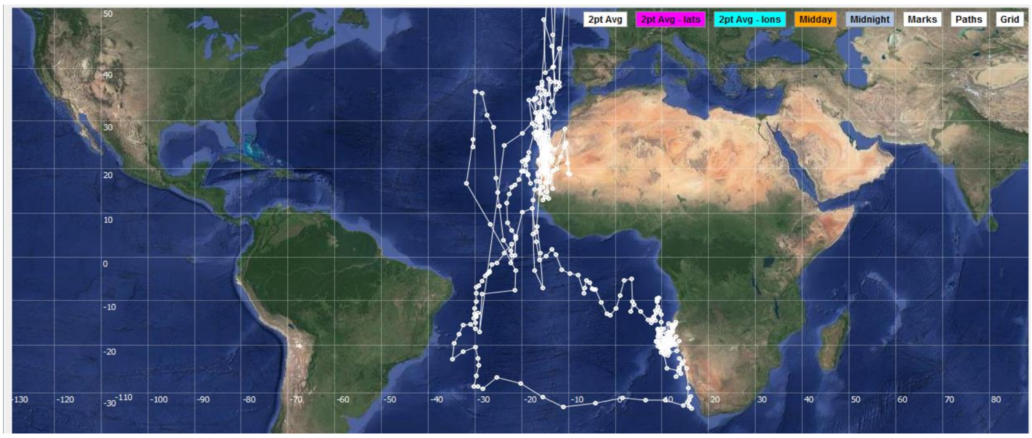


Figure 7-Example of a migratory path created by Intiproc software© with data recorded by a geolocator from one individual wintering in Benguela current

Wintering locations were classified according to the known wintering locations chosen by Cory's shearwaters' individuals mentioned above (Dias *et al.*, 2011). The mean duration of return migration was calculated based on the period of days since an individual left wintering site and got back to the colony. A Pearson's Chi-Square was carried out to see if there was any association between sex and wintering location. All the procedures were made using SPSS statistics 24. The considered significance level was 0.050 and means are represented with the respective standard error (mean ± SE).

Geolocators recovery rate was 84% and data was successfully retrieved for 142 individuals, 94 of it were to males and 41 were females (the sex was unknown for 7 individuals from the total). The average migration duration from the predominant wintering sites back to the breeding colony (nest) at Selvagem Grande was 28 days ±10 days. Most individuals left the wintering site at the beginning of February and arrived at the colony in the middle of March.

Geolocators effects were believed to be negligible according to a previous study done with Cory's Shearwaters by Igual *et al*. (2005). In this case a good indicator of negligible effects was the 84% deployment rate.

Wintering locations matched the ones reported in previous studies. Most individuals migrated to south, to the African shore waters: Benguela current and Agulhas current. The designation "Cabo" was used when it was not clear if the individual spent the winter in Agulhas or in Benguela. Some individuals remained in the Canary current region during winter and others, very few, went to Northwest Atlantic, Central South Atlantic and Brazilian current. As it is shown in figure 8, Benguela was the wintering site where the majority of individuals went (64 individuals, 45.1%), making southern Africa the most common wintering site, with 108 individuals (76.1% of the total) spending winter. 19 individuals, all male, spent the winter in the Canary current (13.4%). Very few individuals spent winter in South Atlantic (4 individuals, 2.8%), Brazilian current (8 individuals, 5.6%) and Northwest Atlantic (3 individuals, 2.1%). Because of that, these locations were excluded from most statistical analysis.

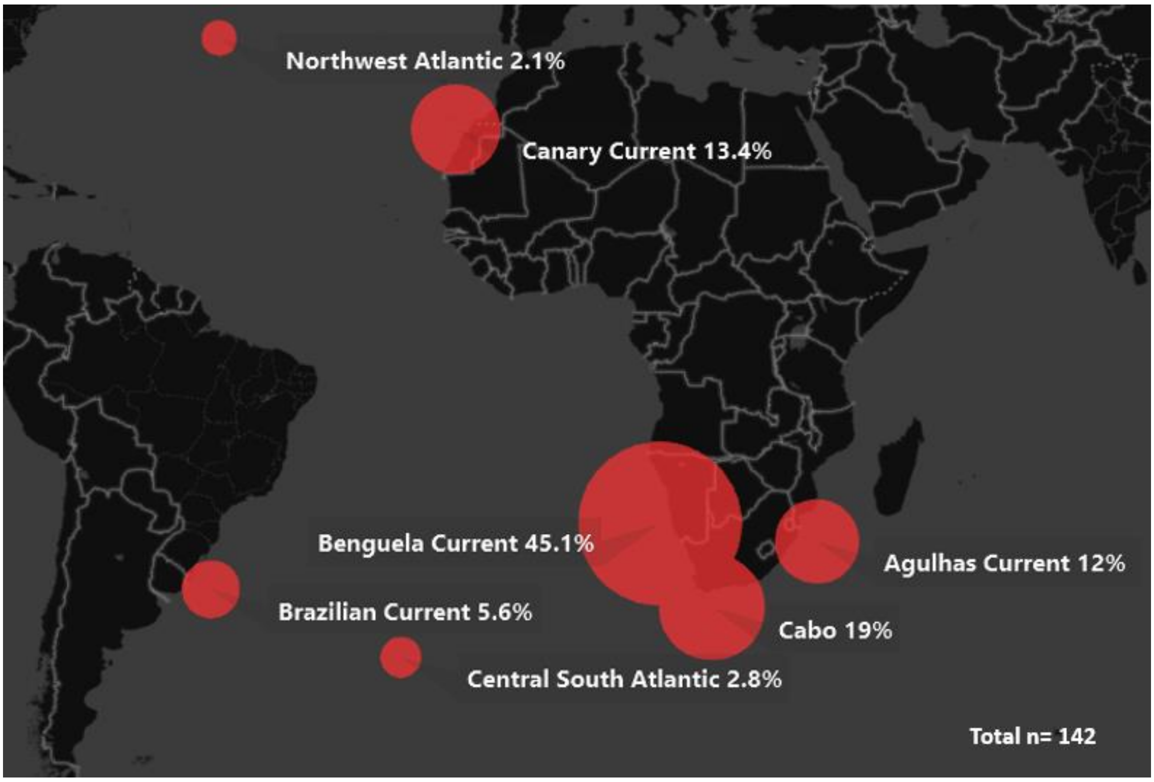


Figure 8-Percentage of Cory's shearwater individuals wintering in each wintering location

When looking to the data separated by sex, there is significant difference between females and males distribution along wintering sites ($\chi^2(1) = 19.467$; $p < 0.001$, n=121), with Agulhas being the only place with an higher number of females comparing to males (11 females (64.7%) and 6 males). Canary Current showed no female attendance and all the other wintering locations had a higher percentage of males. If doing the analysis excluding Canary Current there is also a significant difference between sex distribution among southern Africa locations ($\chi^2(1) = 10.010$; $p = 0.007$, n=102). The percentages of male and female individuals are represented in figure 9. Looking to each sex data, it is possible to see that the majority of males (46.8%) and females (34.1%) went to Benguela. Amongst females, 26.8% went to Agulhas current, while no females wintered in Canary current.

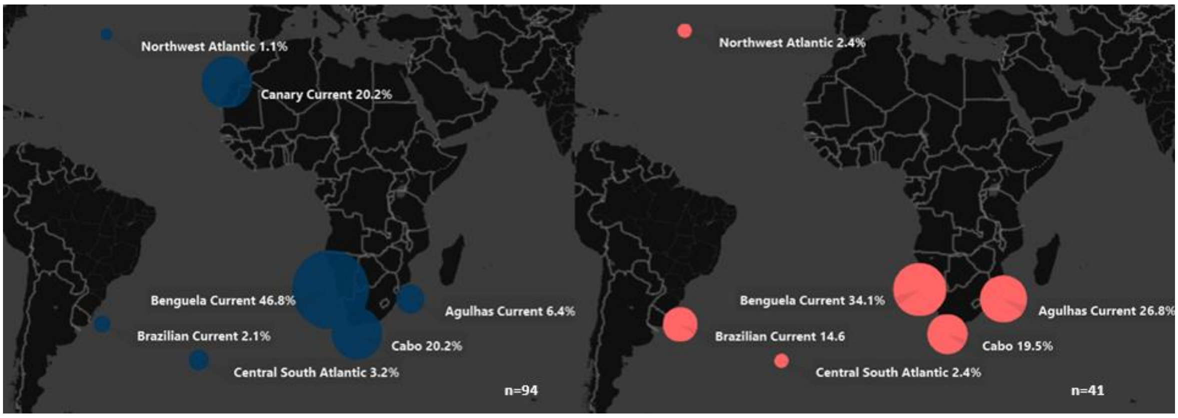


Figure 9-Percentage of the distribution of each sex Cory's shearwater individual across wintering locations (males represented in blue on the left; females represented in pink on the right)

CHAPTER 3 – RELATIONSHIPS BETWEEN WINTERING LOCATIONS, ARRIVAL DATES AT THE COLONY, BODY CONDITION AND REPRODUCTIVE SUCCESS

3.1 Wintering grounds' carry over effects

Conditions experienced on the wintering grounds may influence an individual's body condition (Dias *et al.*, 2011), which can in turn cause variability within a population in individual fitness and performance (Newton, 2008). It can also influence the arrival date at the breeding grounds and reproduction (Mckinnon, 2015). The timing of annual cycle events which can be influenced by the distance of the wintering ground can also have major consequences for breeding success (Kokko, 1999; Newton, 2008).

Understanding the consequences of carry over effects from wintering grounds is important for understanding the mechanisms regulating populations dynamics in migratory birds. This is difficult to assess due to the large spatial scales associated with migrant birds' lifecycles, number of potential ecological constraints and the individuals' variety of responses (Hobson & Man, 2013). It is postulated that individuals should select and choose specific wintering sites that increase their body condition and fitness through effects on survival and on reproduction during the following breeding season (Sedinger *et al.*, 2017).

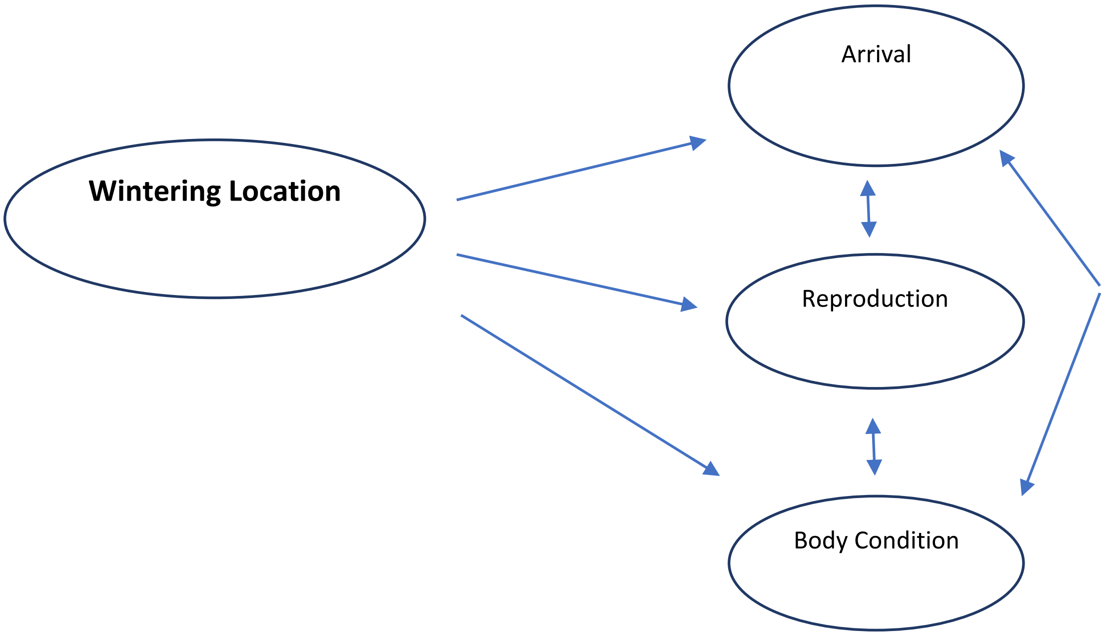
The state on which individuals arrive and the time they arrive at the breeding site will influence the onset of breeding events. Arrival time can be a crucial for the annual cycle, and can even act as a bottleneck factor (Buehler & Piersma, 2008; Rappole, 2013). Long distance migrants tend to arrive later than short distances ones (Hubálek, 2003; Tryjanowski *et al.*, 2005). Arrival dates within a species tend to be during a determined period of time, so they can meet a particular phase of spring phenology. One known migratory strategy suggests, as it was previously referred, that early arrival in breeding grounds is important to the maximization of body condition and reproduction (time-minimization migratory strategy (Catry *et al.,* 2013)) and that delays in a determined part of annual cycle may affect the subsequent ones (Alerstam, 2011; Catry *et al.*, 2013). Although at first sight early arrival brings fitness benefits and advantage in reproduction it also has some risks associated. Individuals arriving earlier in spring can face harsh atmospheric conditions while in migration. This harshness of atmospheric conditions has more effect in terrestrial birds, but can also be felt, with less intensity, in seabirds (Brown & Brown, 2000; Rappole, 2013). Arrival time can vary within a population but also between sexes, depending on species reproductive behaviour (Helm *et al.*, 2006). The most common strategy is called protandry and consists in adult males arriving to breeding colonies earlier than females (Chapman, 1894). Protandry leads to many adaptations, one example of that is partial migration, which was referred in the previous chapter. In this kind of migration, a number of adult males stays near the breeding territory during wintering time, while others and females migrate to other far way locations (Rappole, 2013). Several hypotheses have been proposed to explain early arrival by one sex versus the other at breeding sites. Kokko *et al.* (2006) hypothesise that sexual selection may be the driving mechanism behind early male arrival. This does not explain why females do not arrive early as well, since they can also need to compete for territories and highest-quality males (Rappole, 2013).

Reproduction decisions are extremely important in long lived species like this. If in poor condition, individuals tend to restrain the investment in current reproductive attempts in order to maximize the probability of future reproductive events (Arizmendi-mejía, 2013). Another factor that can influence reproduction and fitness (fecundity and survival) is body condition (Rappole, 2013). Some long term studies in migrant seabirds indicate a contribution of individuals' body mass to reproductive success (Gelbach, 1989; Mills, 1989), although in other species there was no evidence that body mass influenced fecundity and survival (Chastel *et al.*, 2014). Only few studies have focused in body condition affecting reproductive decisions in long-lived seabirds, like pelagic seabirds (Chastel *et al.*, 2014). This group of birds, for example Cory's Shearwaters, can be a good model for studying body condition effects on reproduction because they tend to not compromise their survival (Chastel *et al.*, 2014).

Studying the relevance of factors like arrival time and body condition, depending on the chosen wintering location, provides important information that allows testing if the decision to breed depends on this choice (Drent & Daan, 1980). It also allows investigating if the choice of the wintering location has a major role in populations dynamics.

3.2 Objectives

The main goals were to assess the possible results of "carry-over" effects from different wintering locations, as well as investigate the relationship between wintering locations and arrival dates at the colony, reproduction and body condition, as well as the relationship between those factors.



3.3 Material and Methods

3.3.1. Sampling

Between 2nd of March and 12 of April of 2017 all the study nests in Selvagem Grande were checked on a daily basis, in order to sample individuals on first arrival to the colony from migration. Individuals were weighed to the nearest 10 grams with a Pesola spring balance. Beak measurements to infer body condition and sex, and wing length (maximum flattened cord) were taken (Granadeiro, 1993; Gómez-Díaz & González-Solís, 2007) in case this information was missing from the population's individuals database. Arrival dates refer to the day that individuals arrived at the nest and were confirmed through the geolocators' data. Wintering grounds were also determined from geolocators' data (previous chapter). Reproduction success data was gathered during 2017's breeding season by checking the occupation of nests in June, the presence of an egg, and the presence of a chick during July.

3.3.2 Data analysis

Data normality was assessed by Shapiro-Wilk normality test.

Arrival dates at the breeding ground (“Arrival Dates”) were converted into numerical Julian dates. The difference in arrival dates for males and females was calculated from the difference between the means of arrival day for each sex. To assess differences in arrival dates between sexes a Mann-Whitney test was carried out. This test was also done to assess differences in arrival dates from each wintering locations for each sex.

Body Condition was estimated through a body condition index. A factor analysis was made using the principal components method to estimate PC1 scores, representing body size. The variables used to make these scores were wing length, and beak measures (culmen length, bill height at base and bill height at gonys). After obtaining the body size scores, a generalized linear model was carried out in order to create a body condition index with the residuals from the regression between body size scores and weight, with sex as a factor.

Reproduction success was measured in terms of successful laying and successful hatching. Successful laying was assessed by the presence of an egg in the nest and successful hatching was assessed by seeing if the egg had hatched or not. The differences in reproductive success l laying and hatching) between males and females were studied using a Fisher’s exact test, with the variables being sex and 0 or 1 representing individuals with and without an egg, respectively, and individuals with an egg that did or did not hatch, respectively.

A Spearman's rank-order correlation was run to determine the relationship between arrival dates and body condition, being these two the variables in test.

A binary logistic regression was performed to investigate the effects of arrival date on laying success, the latter one being the dependant variable. The difference in arrival dates between individuals that successfully laid and that did not was calculated between both means and statistically tested with a t-test. Differences in reproductive success according to body condition were assessed by a Mann-Whitney test, with laying success and body condition being the variables in test and also hatching success and body condition.

To assess reproductive success (laying and hatching) in individuals from different wintering locations a Pearson’s Chi Square test was performed. A Fisher’s exact test was used to investigate laying success between Southern Africa locations and Canary current.

Differences in body condition and arrival dates depending on wintering locations were investigated using Kruskal-Wallis test, with the groups being the different wintering locations and body condition and arrival dates the tested variables. Post hoc tests showed the specific differences between each location.

All the tests were repeated separately for each sex and for each wintering location, unless stated otherwise. All the statistical analysis were done using SPSS Statistics, with a significance value of 0.050.

3.4 Results

3.4.1 Arrival dates at the colony

Arrival dates at the colony of a total of 152 individuals vary between 23.02.2017 and 09.04.2017. Its distribution is represented in figure 10. The dates registered may be slightly influenced by our departure date from the study site, since a few birds carrying GLS arrived after the field work ended and were encountered later in the year, although, and according to the distribution curve, these individuals were in a small number (n=6). Data from these birds are not included in this study. 50% of individuals arrived between 07.03.2017 and 18.03.2017.

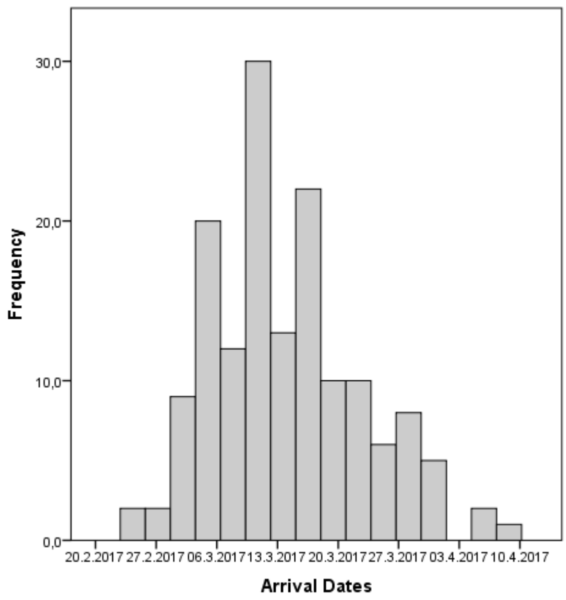


Figure 10-Cory's shearwater's arrival dates distribution along sampling period in Selvagem Grande

Males arrived significantly earlier than females when considering all locations (U= 2.780; p=0.016, n=121) (figure 11), on average 4 days earlier. When considering only individuals from southern Africa locations, all of them combined, there are also differences in arrival dates (U= 1.424; p=0.041, n=102).

Doing the analysis for males and females separately for each wintering location of southern Africa the results showed that there are no significant differences between arrival dates from males and females (Benguela U= 1.506; p=0.202, n=58; Cabo U= 70.500; p=0.769, n=27; Agulhas U= 43.500; p=0.290, n=17).

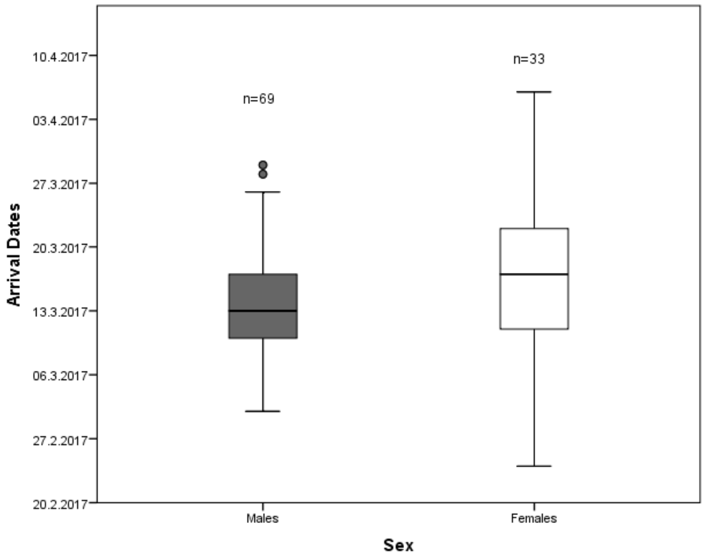


Figure 11-Differences in arrival date frequencies between sexes from Cory's shearwaters' individuals wintering in southern Africa locations

3.4.2 Reproductive success

Reproductive success was defined by two categories: whether the individuals successfully laid an egg, "laying success"; and among the individuals that laid an egg: whether individuals successfully hatched, "hatching success". Data on fledging success was unavailable during the analysis of this study.

In a total of 152 individuals that were tracked, 73.7% were incubating an egg in June. Between the birds that had eggs, 78.6% of them successfully hatched a chick. Laying success tested between males and females showed a significant association between the presence of an egg in the nest and sex (Fisher's Exact test χ^2 = 6.658, p = 0.007, n=145). 68.3% (n=101) of males had eggs in the nest while 88.6% (n=44) of females had eggs in the nest. In figure 12 it is possible to observe the high percentage of females with an egg in the nest (successful laying) and low percentage of females with no egg in the nest (failed laying). When compared to males, the difference in percentages between males with and without an egg in the nest was smaller.

Hatching success results did not show any interesting or significant differences amongst wintering groups and when comparing with the other variables in study and overall were high. Because of that the results showed are only related to laying success.

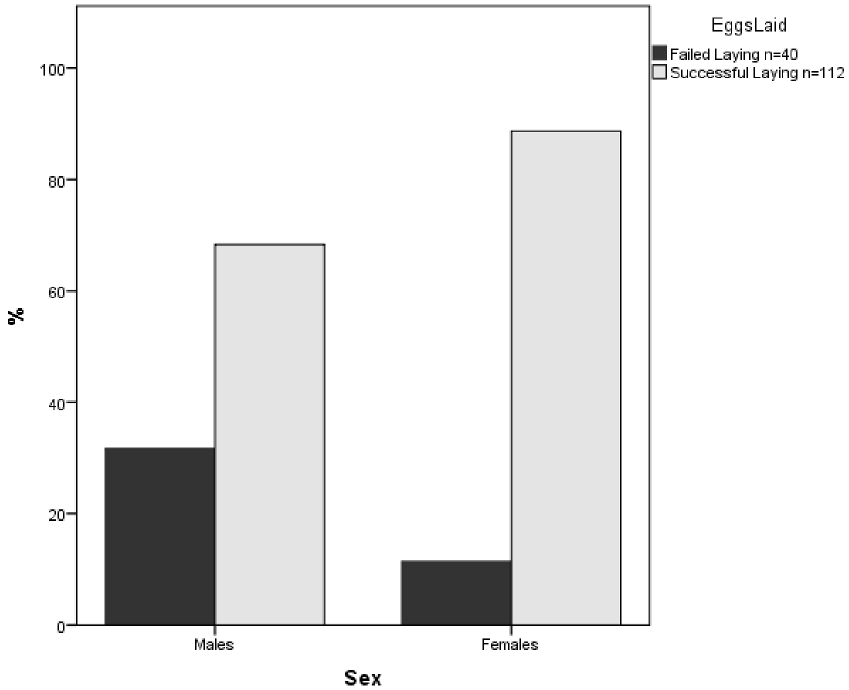


Figure 12-Percentage of laying success (egg in the nest) between sexes of Cory's shearwaters' individuals

3.4.3 Relationship between Arrival dates and Body Condition

There was no statistically significant correlation between arrival dates and body condition (r_s = -0.104, p = 0.237, n=131) (figure 13). There was also no correlation between arrival date and body condition either for males (r_s = -0.096, p = 0.375, n=88) nor females (r_s= -0.077, p = 0.623, n=43). There was no correlation amongst males from Canary current (r_s= -0.040, p = 0.883, n=19) nor amongst males from Southern Africa (r_s = -0.214 p = 0.104, n=69).

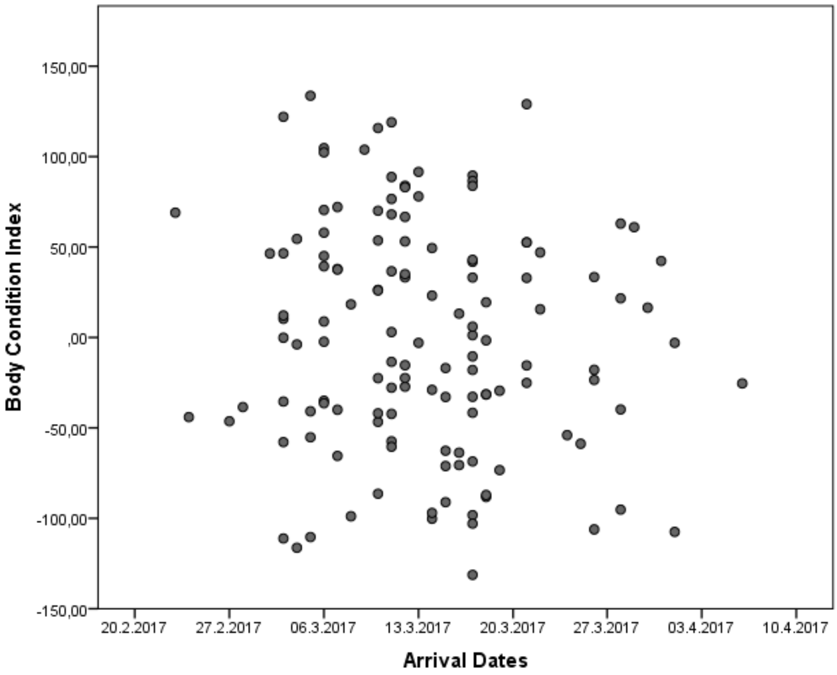


Figure 13-Relationship between Cory's shearwaters' body condition and arrival dates

3.4.4 Relationship between Arrival Dates and Laying Success

The binary logistic regression model results showed no relationship between arrival dates and laying success (R^2=0.007, χ2 = 2.399, p =0.450, n=121). The results are represented in figure 14. According to the average arrival dates, individuals that successfully laid arrived 1 ± 1.6 days earlier than individuals who failed laying.

When doing the analysis sorted by sex, the results were similar (males: (R^2=0.026, χ2 =315.289, p =0.202, n=88); females: (R^2=0.014, χ2 = 13.795, p =0.623, n=33)).

Amongst males from Canary current and from southern Africa, arrival dates also did not have any effect in laying success (R^2=0.078, χ2 = 0.470, p =0.310, n=19 and R^2=0.045, χ2 = 16.014, p =0.141, n=69 respectively).

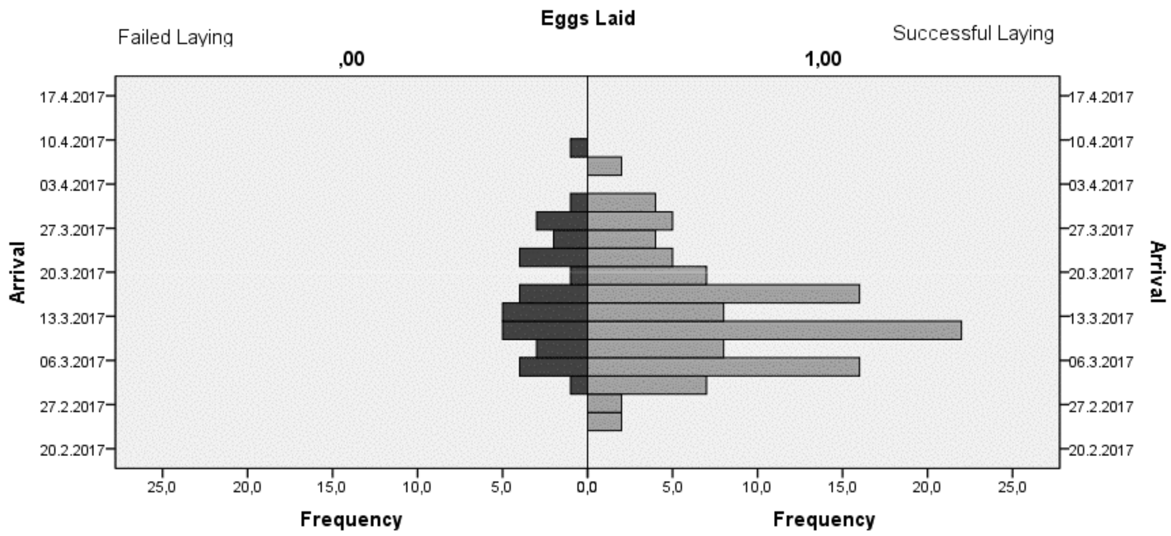


Figure 14-Frequency of Cory's shearwaters' laying success along arrival dates for both sexes

3.4.5 Relationship between Body Condition and Laying Success

Body condition was not different between individuals with and without eggs in the nest (U= 1 830.0; p=0.259, n=131) (figure 15). It was also not significantly different between males with a partner that successfully laid an egg or not (U= 977.0; p=0.220, n=88) and between females that successfully laid an egg or failed (U= 92.0; p=0.927, n=43).

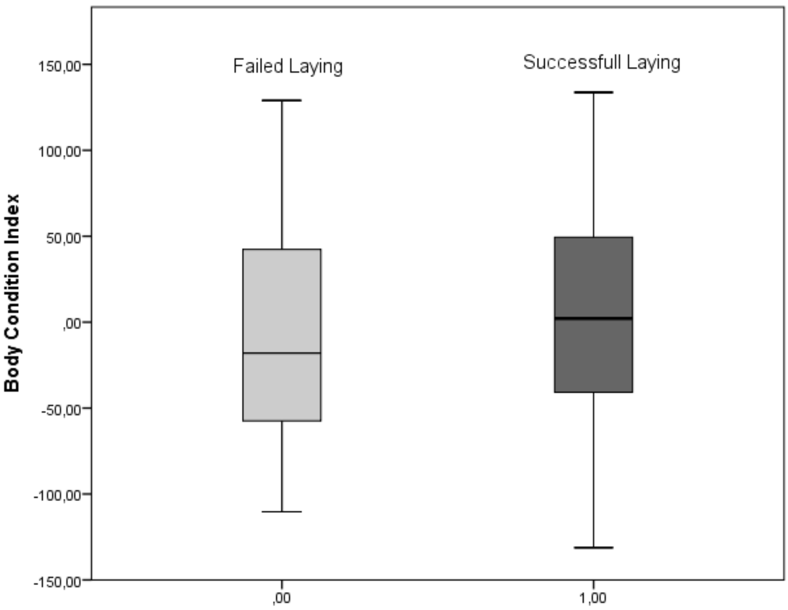


Figure 15-Relationship between Cory's shearwaters' body condition and laying success

3.4.6 Relationship between Wintering Sites and Laying Success

A greater percentage of individuals wintering in the Agulhas Current had an egg at the following breeding season (82.4%, n=17) compared to the other locations. The lowest percentage of successful laying was recorded for birds wintering in the Canary Current, (57.9%, n=19). However according to Pearson's Chi Square test there is no statistically significant association between wintering sites and the likelihood of laying (χ^2 = 3.778, p = 0.286, n=127). These results are represented in figure 16.

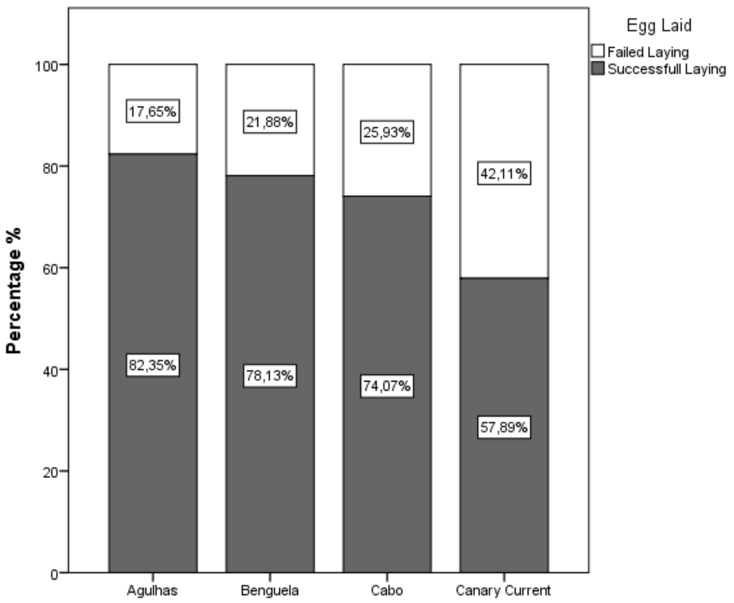


Figure 16-Percentage of Cory's shearwaters' successful and failed laying in different wintering locations

If doing the tests only with two groups (Southern Africa locations and Canary Current) the differences are more pronounced (Fisher's exact test χ^2 = 3.389, p = 0.086, n=127) but still not significant.

Southern Africa individuals (n= 108) have 77.8% of laying success and Canary Current's (n=19) 57.9%.

Doing separate analysis for males, 57.9% of the individuals wintering in Canary Current had eggs while 75.4% of the males that wintered in Southern Africa locations had eggs (figure 17). There was still no significant association between groups (Fisher's exact test (χ^2 = 2.235, p = 0.157, n=88)).

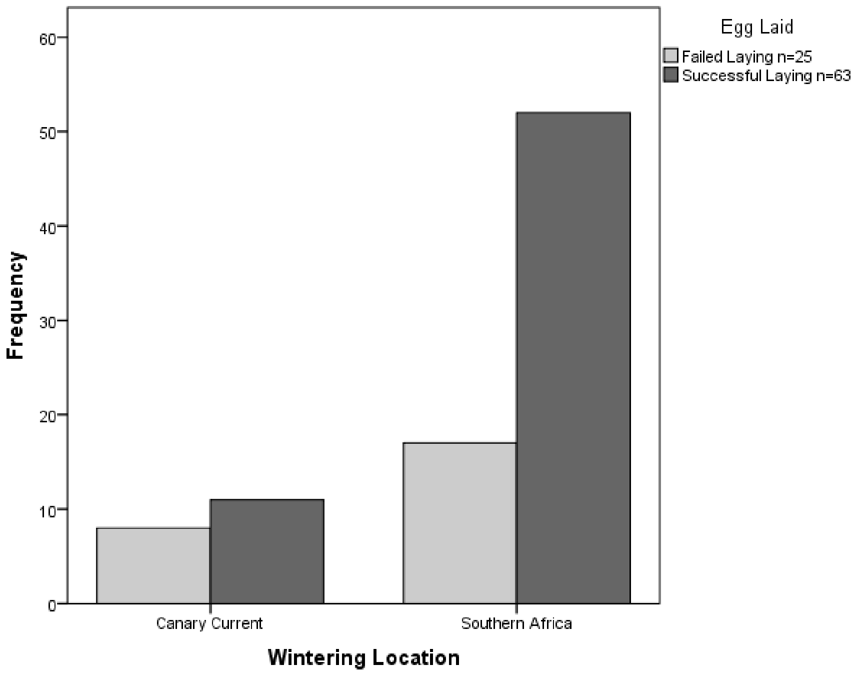


Figure 17-Successful laying frequency between male Cory's shearwaters wintering in southern Africa locations and Canary current

3.4.7 Relationship between Wintering Site and Arrival Dates

The wintering site with higher range of arrival dates is Benguela, which is expected since it is the place with the higher frequency of individuals. Individuals wintering in southern Africa locations seemed to arrive later to the colony (figure 18). The results indicate that birds spending the winter in the adjacent Canary Current region are among the first to return to the breeding site (on average, Canary current individuals returned 7 ± 1 days earlier that individuals from Southern Africa locations).

When analysing males there are also differences between groups (Kruskal-Wallis H = 11.530, p = 0.009, n=88). It is possible to see that Canary Current male individuals return earlier, with Kruskal-Wallis post hoc showing significant differences in arrival dates between birds wintering in the Canary Current and Cabo (H = 26.789, p = 0.001, n=38). For females (none wintering in Canary current), there was no significant difference in arrival dates between individuals wintering in distinct locations (Kruskal-Wallis (H = 0.982, p = 0.612, n=33). The results are presented in figure 18.

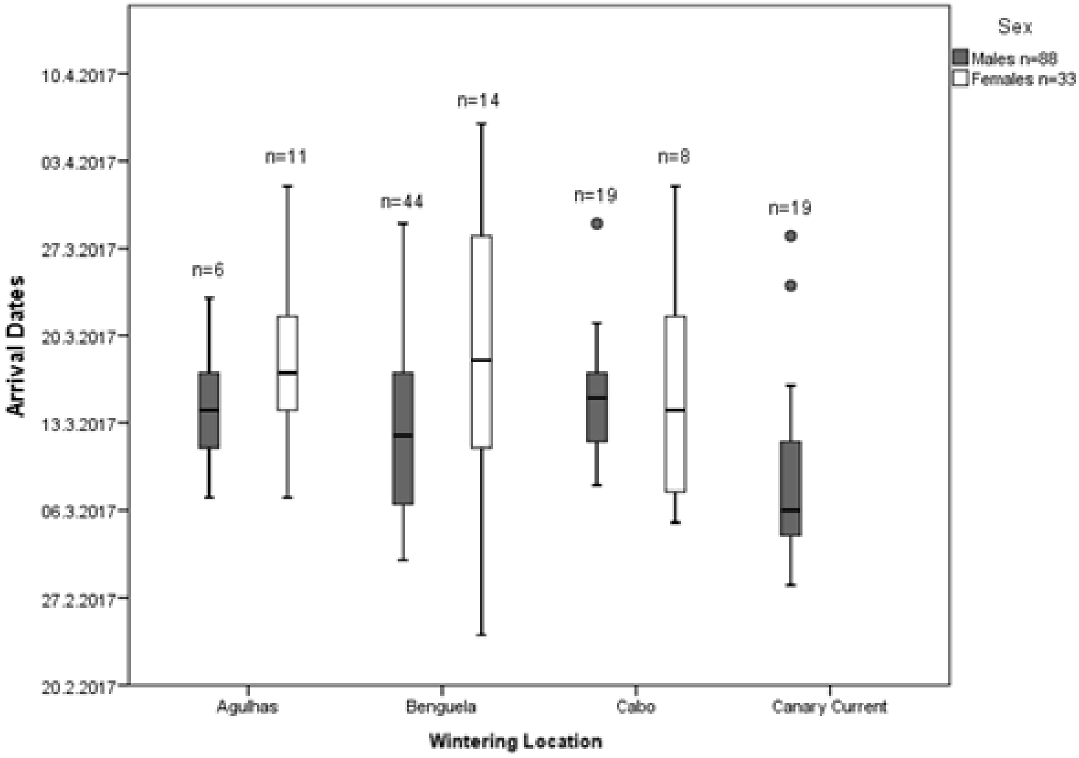


Figure 18-Differences in Cory's shearwaters' arrival dates between wintering locations

3.4.8 Relationship between Wintering Site and Body Condition

Kruskal-Wallis test between male individuals showed that there were no significant differences in body condition amongst wintering locations groups ($H = 6.855$, $p = 0.077$, $n=88$) but apparently, Canary Current males have lower body condition. Between female individuals there were no differences in body condition amongst individuals wintering in different locations either ($H = 0.686$, $p = 0.716$, $n=32$) (figure 19).

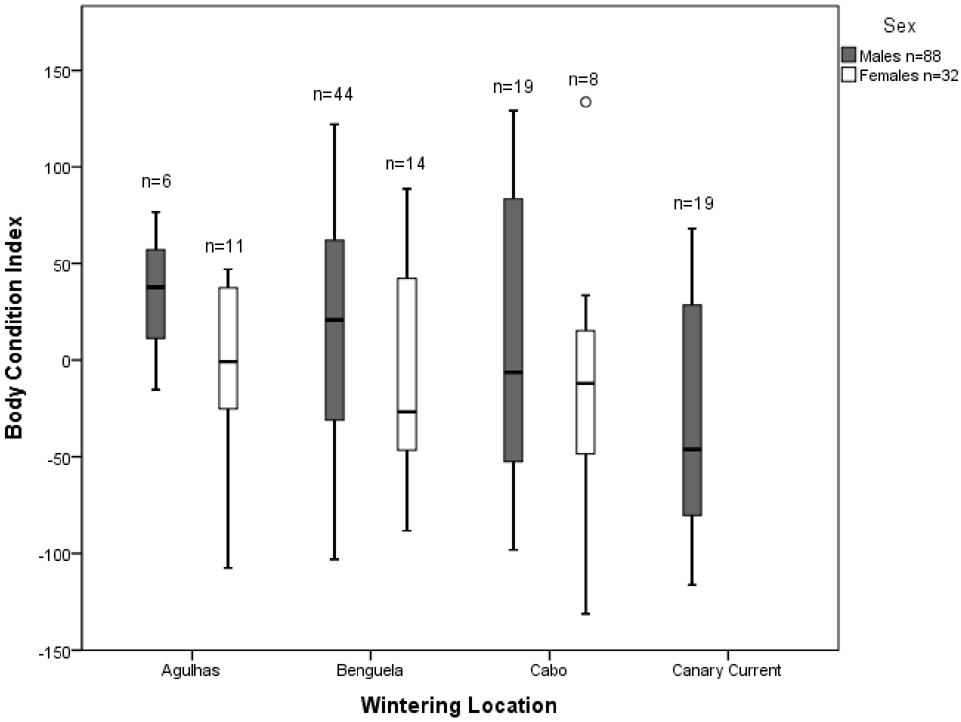


Figure 19-Variation of Cory's shearwaters' body condition index amongst wintering locations

3.5 Discussion

The results obtained from this study showed that, amongst wintering locations, Canary current was the one with individuals arriving earlier, all of them being males. However, there were no differences in arrival dates at the colony between sexes amongst the individuals from southern Africa locations. Relative to reproduction, overall laying and hatching success were close to values recorded for other seabirds (Weimerskirch, 1992). Apparently, there was an association between laying success and sex, with females being the ones with higher success rate comparing to males. Also, only between males, laying success was higher for males wintering in southern Africa locations (75.4%) than in males wintering in Canary current (57.9%), although this difference was not statistically significant. Laying success did not increase with early arrival dates at the colony as it would be expected. According to the results body condition did not have any relationship with reproduction and arrival date but it seemed slightly lower for males wintering in Canary current, in comparison with the other males.

Males are expected to arrive earlier than females, since they are more likely than females to spend the winter in the Canary current, closer to the breeding colony (Catry *et al.*, 2005). This behaviour is seen as an advantage, since males are the sex which needs to secure a good nesting site, against competitors (Catry *et al.*, 2005). In the present work, the fact that only males partially migrated is coincident with previous studies referring to individuals remaining resident during winter, in its majority males (Pérez *et al.*, 2014), with the larger sample size of resident birds analysed here (all of them being males) affording a better description. When observing individuals that wintered in southern Africa locations, which was the majority of individuals, males did not arrive earlier than females. This goes against what is expected, since males are more pressured to return earlier to the breeding colony in order to secure a good nest site (Catry, 2005). However, if females also fight for better nests and partner like it is stated by some literature for other seabirds (Rappole, 2013) it would make sense that they arrive at the same time than males. While in Selvagem Grande during field work there were actually indices of female/female fightings occurring, but this requires more investigation. Despite that, the fact that individuals would arrive earlier as a reproductive investment can be contradicted by the apparent lack of influence of earlier arrival on laying success in this work, which is not in agreement with other studies of migratory birds (Newton, 2008). It also does not match the arrival-time hypothesis to explain differential distance migration of sexes, since there is a tendency for individuals arriving early to the colony as a reproductive investment. The sooner the individuals arrive at the colony (in these case, males), the sooner they will secure a nest and avoid confrontations, increasing the odds of successful breeding (Pérez *et al.*, 2014).

Despite the overall high percentages of laying success in all wintering locations, similar to previous results (Weimerskirch, 1992), males wintering in Canary current apparently had lower laying success comparing with the ones from southern Africa locations, although this relationship in not statistically significant. Laying success was also higher in females comparing to males. The males from Canary Current stayed resident, as a reproductive investment, in order to arrive earlier to the colony. However, this investment seems to have failed since it is suggested that males wintering in Canary current had a slightly lower laying success. These two facts can indicate that male individuals outnumber the number of females and although the effort of being in Canary current during winter to secure a nest earlier and enhance the chances of breeding, this had no effect possibly due to a lack of females, since a sex ratio bias can lead to a lower fecundity among individuals of the more abundant sex (Weimerskirch *et al.,* 2005). Little is known about sex ratio variations in monogamous species, especially long-lived species where sex ratio variations are likely to occur due to age-related differential reproductive effort or sex-specific differential mortality (Weimerskirch *et al.,* 2005). In this case, a possible male biased sex ratio could be related to the age of the individuals, with one sex having older or younger individuals. The possibility of a male biased sex ratio can also be an indicative of poorer quality of Agulhas current, since, as it was shown in the previous chapter, this is the location chosen by a great part of females and where the water temperature is known to be increasing, in a higher rate when comparing to other regions, since 1980's due to climate change (Rouault *et al.*, 2009). This has negative effects on its productivity levels and, consequently, Cory's Shearwaters' prey can become scarce, reflecting in populations decrease (in these case, of females). Although, if this was the case, it would also be expected that females' body condition was lower, which did not happen. However, the higher attendance of females in Agulhas can happen because of its lower energy requirements or different prey choices due to different size and requirements on return to the colony, allowing them to exploit Agulhas current better than males. Despite that, these assumptions are speculations that would require further investigation.

Another factor that it is known to influence reproductive success in several avian species is pre-breeding body condition (Arizmendi-mejía *et al.*, 2013), although the influence of body condition on reproductive success has not been clearly established in pelagic seabirds (Chastel *et al.*, 2014). Body condition measured after arrival at the breeding grounds can be an indicator of the quality of the wintering ground (Arizmendi-mejía *et al.*, 2013). In this study, body condition was not significantly different between arrival dates and wintering locations and also when comparing with laying success. Regardless of that, it seemed to be slightly lower for male individuals wintering in the Canary current, when comparing with other males. Lower body condition could be an effect of a poorer quality of this wintering area, in terms of productivity and climate conditions (Kämpf & Chapman, 2016), only being chosen by males with the purpose of arriving earlier to the colony as a reproductive investment. Negative impacts of harsh winters are often reported in studies of migratory birds, although the persistence of these effects in subsequent seasons is rarely investigated (Clausen *et al.*, 2015). The fact that males wintering in Canary current had a slightly lower laying success when compared with the other males can be due to the fact that these males that partially migrate and stayed in a harsher wintering location, consequentially had a lower body condition by the time of arrival to the colony. Despite that these males were the first ones to secure a nest, due to their poorer body condition they could have lost their nest or mate when competing with other males, with better body condition and who arrived latter.

Other explanation is that these males with lower body condition can be restraining their current breeding attempt in order to maximize the probability of future reproduction (Arizmendi-mejía, 2013).

Amongst males that migrated to southern Africa locations, no pattern previously described for migratory seabirds relatively to body condition and arrival at the colony was shown. According to Chastel *et al.* (1995) and Szostek *et al.* (2015), males in good body condition tend to arrive earlier to the colony, but in this study, between individuals wintering in southern Africa locations, there was no relationship between body condition and arrival date at the colony. In this case Canary current males were the ones arriving earlier but also the ones with lower body condition, however, because these males partially migrate, they are not good objects of comparison.

The possible male-biased population and also the lower body condition from Canary current individuals can be “carry-over” effects as a result of the chosen wintering location, they can also be taken as clues for environmental problems in the regions were those individuals spent the winter. A lower body condition present in males wintering in the Canary current can be affecting its reproductive success. These problems may be due to climate change or anthropogenic pressures, like fisheries and pollution. Also, the results from the present work can be of value when taking measures of conservation towards marine species, for example while delimitating marine protected areas; and/or political measures about environmental uses.

CHAPTER 4 –MERCURY CONTAMINATION ACROSS WINTERING GROUNDS

4.1 Introduction

Mercury concentrations have increased in many regions of the globe (Monteiro & Furness, 1995; Harris et al., 2007). It is thought that along the Atlantic Ocean mercury atmospheric concentrations increased at a rate of 1% each ear during the 1980's and nowadays they are believed to be reaching a stable level (Lindberg, 1987: Monteiro & Furness, 1995; Pacyna *et al*., 2006).

Concerns about the mercury effects as a global contaminant have increased in recent years (Pacyna *et al*., 2006; Seewagen, 2010; Gworek *et al*., 2016). It is now considered one of the most serious pollutants and it can be highly toxic. It tends to bioamplify through food chains and accumulates at higher levels, like most of trace metals (Bryan, 1979; Monteiro *et al.*, 1995; Bank, 2012; Driscoll *et al*., 2013).

The inputs of mercury in the environment are in is majority caused by humans (Monteiro & Furness, 1995) but background levels are present naturally in food webs. Mercury is discharged into the environment by volcanos and it is naturally present in the Earth's crust. One of the challenges faced today is to identify mercury levels caused by human activity against a background of variable natural levels (Newman, 2014). However, mercury concentrations have increased considerably due to the industrial development and increase in combustion of fossil fuels. It is released to the atmosphere mainly from coal and oil product combustion in utility, commercial or residential boilers; cement production in wet and dry rotary kilns; primary and secondary lead and zinc production; pig iron and steel production; caustic soda production; mercury and gold production and waste disposal (Pacyna *et al.*, 2006). The major sources and transport processes of mercury to the open ocean are atmospheric deposition and runoff from coastal and freshwater systems followed by advection offshore (Bank, 2012).

Although most of mercury releases came from human activities in the surrounding areas to the polluted site, in certain places, after it enters the atmosphere it is spread over very large areas (Pacyna *et al.*, 2006; Newman, 2014). For what is known, atmospheric mercury emissions occur in larger numbers in the northern hemisphere comparing to the southern hemisphere, resulting in higher mercury levels registered in the north (Lamborg *et al.*, 1999; Masekoameng *et al*., 2010; Weigelt *et al*., 2015). Nowadays, southern hemisphere is showing an increase in mercury levels and that may be due to the industrialization of developing countries from this part of the globe (Newman, 2014).

The major part of the mercury (Hg) released to the environment is inorganic, but a fraction of it is converted to its organic form, which is methylmercury (MeHg). Methylmercury is the one that accumulates through aquatic food webs and the one that is most present in wildlife (Harris at al., 2007).

In recent times a bigger effort has been made in order to better understand mercury dynamics in seabirds (Thompson, 1990; Monteiro & Furness, 1995; Kojadinovic *et al*., 2007; Carravieri *et al*., 2014; Bond *et al*., 2015). This is providing a good basis in a way of using them as monitors of geographical, temporal and global patterns of mercury contamination in the marine environment (Monteiro & Furness, 1995; Seewage, 2010). When looking through the history, seabirds are among the first victims of known episodes of mass contamination by mercury, like in the Minamata incident in Japan, for example. However, this did not result in comprehensive studies of mercury toxicity for these species and there is still a lack of data on the negative effects in wildlife (Monteiro & Furness, 1995; Scheuhammer *et al.,* 2015). Mercury levels in seabirds are known to be high and above the thresholds of toxicity in humans, which can suggest a certain level of adaptation developed by these species (Monteiro & Furness, 1995; Scheuhammer *et al.,* 2015). However, information about toxicity levels in wild populations is still lacking (Thompson, 1990; Monteiro & Furness, 1995; Scheuhammer *et al.,* 2015). The high levels of mercury found in some seabirds raise questions about its potential toxicity because these species present mercury concentrations in the range that would cause toxic effects in some terrestrial bird species. Because of that lack of information there is a need to study the effects of this contaminant in wild individuals, in a way that its toxic effects can be better assessed (Monteiro & Furness, 1995; Harris *et al.,* 2007; Seewagen, 2010). It is also important to assess if besides its toxicity levels, mercury can somehow influence any part of the individuals live cycle and population dynamics.

4.1.1 Seabirds as bioindicators

Bioindicator is an organism (or a part of an organism, or a community of organisms) that responds predictably to contamination, providing information on the quality of the environment (Zillioux & Newman 2003; Markert *et al*., 2003). The response to contamination can be of physiological or ecological organization, as well as of molecular level or population and community level (Harris *et al.*, 2007). A bioindicator can be a biomonitor if it provides quantitative information of the aspects of the quality of the environment (Markert *et al*., 2003). Bioindicators can be used for various reasons. It can be to measure the level of environmental contamination, or the rate of change in contamination, to assess the rate of release from a pollutant into the environment, to assess the biological effects of pollutants on species or communities or even to assess the hazards to humans (Furness, 1993; Newman, 2014).

Seabirds are good bioindicators and biomonitors of the contamination status in the environment because they not only reflect the presence of the contaminant of interest, but also integrate it over time and space, and local, regional and global sources (Harris *et al.*, 2007).

Most environmental contamination studies emphasize the use of sedentary invertebrate animals and by comparison, seabirds, especially long distance migrants, have some disadvantages. Long distance migrants like procellariids are very mobile, and because of that contaminants will be picked up from a wide geographical extension; they live long lives, what can cause pollutants to accumulate over time in complex ways; their physical complexity is much higher than in invertebrates; and they usually are more difficult to sample, adding to it ethical issues related to sampling (Furness, 1993; Furness, 2012). These seemingly disadvantageous characteristics can be desirable traits at times. The integration of contaminant levels over greater areas or time-scales can be useful if the aim is to monitor over a broad scale and the ranging behaviour of the birds is known. Less sampling may be necessary if birds can reflect contaminant levels in the whole ecosystem or over a wide area (Walsh, 1990; Burger, 1993; Kojadinovic *et al*., 2007). Procellariids, for example, aggregate in colonies, so sampling a large number of individuals also became easier. It allows large numbers to be tracked for less effort than if the breeding populations were dispersed, it allows large quantities of data to be collected from a specific site in a relatively short period of time (Furness & Camphuysen, 1997; Furness, 2012), and, because they show a philopatric behaviour, returning to the same nest site and colony for years, contaminant loads of individuals can be easier studied over time (Burguer, 1993; Furness, 2012). Another possible advantage is that these birds are in higher levels of the food chain, so they can reflect pollutant hazards to humans better than most invertebrates (Furness & Camphuysen, 1997; Hoffman *et al*., 2002). In addition, long distance migratory birds are very popular in the general public, making them a flagship species and a greater object of attention than invertebrates (Furness, 1993).

A good example of a procellariid bird that fits in this description and holds a wide range of sites in its lifecycle is Cory's shearwater (*Calonectris borealis*) (Furness & Camphuysen, 1997), which is the subject species of this dissertation. The knowledge that has been acquired of the general seabird ecology and of the numbers and productivity of many populations also makes them a particularly good choice of bioindicator or biomonitor (Harris *et al.*, 2007).

The selection of a particular species to a particular area of interest must depend on its life history strategy, breeding cycle, behaviour and physiology, diet and habitat uses (Schreiber & Burger, 2001; Harris et al, 2007). Being top-predators of the marine environment and spending most of their lives in the sea, seabirds can reflect contaminant levels as they bioaccumulate up the food chain (Walsh 1990; Furness & Camphuysen, 1997¸ Kojadinovic *et al*., 2007; Furness, 2012). This bioindicator role has helped many times before in the detection of pollution problems. One example of that happened in the southern North Sea, where the first notice of Drin compounds (pesticide pollutants) contaminating the sea was due to the effects seen on local seabird populations (Furness & Camphuysen, 1997). Mercury levels can be examined in seabirds as an indicator of potential harm to the birds themselves and as bioindicators of local marine pollution but also to assess pollution over wide-scale geographical areas (Schreiber & Burger, 2001).

4.1.2 Mercury in Seabirds

As mentioned before, mercury is a non-essential element that bioaccumulates in organisms and biomagnifies through food webs. Both processes are due to the conversion of inorganic mercury into organic forms, mainly methylmercury, in aquatic ecosystems. Although most of the mercury present in the environment is inorganic, methylmercury is the most highly bioavailable and toxic. This kind of mercury is converted from inorganic mercury by certain bacteria and this organic form can be very efficiently assimilated from water or food, being accumulated over the trophic chain (Thompson *et al.*, 1998; Newman, 2009). Because of that, the majority of mercury present in vertebrates is methylmercury. Due to its toxicity, it is associated with adverse effects such as neurological, immunological, physiological and reproductive impacts in both humans and wildlife (Thompson & Furness, 1989; Scheuhammer *et al*., 2015). Some organisms, including marine mammals and few seabirds developed adaptations that allow them to convert methylmercury back to the inorganic form (demethylation), which can be stored as crystals in the liver or kidneys. This adaptation can be important for animals that do not have the ability to excrete methylmercury (Furness, 1993; Schreiber & Burger, 2002). Other animals have the ability to excrete methylmercury through feather or hair, since it naturally binds to growing hair, feathers and also eggs. It connects with sulphur-containing amino acids, which are present in keratin, the main protein constituent of feathers and hair (Newman, 2014). Because of this methylmercury property, when seabirds have high loads of it in their bodies, feathers grow containing correspondingly elevated levels of mercury (Appelquist *et al.*, 1984; Furness, 2012).

Large variations in mercury concentrations between individuals have been attributed to a variety of factors such as migratory habits, body size, life span and moult strategy (Monteiro *et al.*, 1998).

Seabirds, in their majority, do not demethylate mercury because they can excrete it. With the eggs being a way of excretion there is a slight tendency for female birds to have lower levels of mercury in their plumage than males (Burge & Gochfeld, 2002). This difference between both sexes is rather low and usually obscured because mercury levels vary considerably among individuals (Lewis *et al.*, 1993; Bustamante *et al*., 2016).

In laboratory experiments, mercury caused a wide range of reproductive effects including lowered egg weight and shell-less eggs, embryo malformations, reduced hatchability, reduced chick survival and sterility. These possible consequences can interfere with population dynamics (Furness, 1993, Hoffman, 2002). The concentrations often associated with these kinds of lesions are 5 to 65 mg/kg (dry weight) in feathers and 1 to 5 mg/kg dry in eggs (Burger & Gochfeld, 1997).

One obstacle faced to determine the toxicity is that it depends on the dose, form, exposure, species, age, and physiological condition (Eisler, 1987; Schreiber & Burger, 2001).

Mercury levels studies can be targeted towards environmental purposes and studies but also ecology ones, since they can indicate mercury levels in food webs. In Cory's shearwaters, the species studied in the present work, there are some records on mercury accumulation in feathers during the last 150 years and levels are thought to be decreasing or, at least stabilizing in Europe and North America (Monteiro *et al*., 1999; Kojadinovic *et al*., 2007; Becker *et al*., 2016). Despite the information that already exists, few studies reported possible effects in the birds' annual cycle, body condition and reproduction caused by mercury carry over from the wintering areas. As such, mercury toxicity levels in this species and at which concentrations can it lead to negative consequences in individuals is still unknown. In addition, most of the studies are restricted to areas in North Atlantic Ocean. Little is known about mercury pollution and accumulation in seabirds that spend the winter in South Atlantic regions surrounding southern Africa.

4.1.3 Mercury in feathers

Feathers are one of the most popular ways to measure mercury levels, and that is because they act as relatively stable sites for its deposition (Crewther *et al.,* 1965; Appelquist *et al.,* 1984). They are also the major pathway for mercury elimination (70-90%) and accumulation, when comparing with other tissues (Thompson *et al.*, 1990). They can be removed without harm to the bird, and then analysed to give an indication of species' exposure (Schreiber & Burger, 2001). Although feathers seem to be a very appealing method to assess mercury levels, correct interpretations require knowledge about feathers development and chemical structure (Furness *et al.*, 1986; Monteiro *et al.*, 1995; Furness, 2012).

Mercury levels accumulate in body tissues between moults, since most birds undergo active moulting during a determined part of the year (Furness *et al.*, 1986; Lewis & Fumess, 1991; Kojadinovic *et al*., 2007; Watanuki *et al*., 2015). The concentrations of mercury in feathers reflect the contaminant level circulating in the blood at the time of feather growth, and so, the mercury intake from the diet at that time (Furness *et al.*, 1986; Burger, 1993; Lewis *et al.,* 1993¸ Kojadinovic *et al*., 2007; Watanuki *et al*., 2015). Mercury binds to feathers during growth when the feather is connected with the bloodstream through small blood vessels (Burger, 1993; Bortolotti, 2010). The bond between mercury and the feather structure is so strong that it is not affected by a variety of rigorous sample treatments (Crewther *et al.*, 1965; Furness *et al.*, 1986). When the feather is fully formed, the connection between it and blood vessels is shut down, so it becomes physiologically separated from the bird and no more mercury can be accumulated (Denneman & Douben, 1993; Dauwe *et al.*, 2003). Mercury present in a sample of feathers provides an easily-obtainable non-invasive method to measure the mercury burden of a given individual, avoiding the need to sacrifice large numbers of birds, since is rather easy to cut small samples of feathers from live birds and use these samples to measure mercury exposure (Thompson *et al.*, 1998; Furness, 2012; Bustamante *et al.,* 2016).

There are still uncertainties in the interpretation of mercury concentrations in feathers, as a result of a poor knowledge of its physiology and mechanisms affecting mercury deposition (Roque *et al.*, 2016). Some authors state that feathers that go through moulting earlier have highest mercury concentrations as mercury is mobilised from organs where it has been stored (Furness *et al.*, 1986; Braune, 1987). Others state that the previous pattern creates artifacts by ignoring the physiology of feathers (Bortolotti, 2010). These can happen because some elements and compounds are incorporated deeply in the keratin structure while others enter the developing cells in proportion to their abundance in the bloodstream, which occurs with mercury. Because of that, mercury accumulation expressed as concentrations are meaningless due to the varying mass across the feather and with no biological significance (Bortolotti, 2010). Most recently, Roque *et al.* (2016) came to show that the most decisive factor affecting mercury concentrations is feather growth rate. However, intra-study comparisons are still possible when those comparisons are made within the same species and using samples from the same kind of feather between individuals.

Usually, flight feathers are sampled because they are large, easy to identify, robust and easy to treat, and paired with a knowledge of moult order and location of growth can give more fine-tuned information (Furness *et al.*, 1986; Furness, 2012). Nevertheless, the relationship between concentrations in feathers and in other tissues is poorly documented (Thompson *et al.*, 1990), reflecting the lack of multi-tissues studies (Furness, 1993, Furness, 2012).

Because all mercury measured in feathers is methylmercury it is extracted and guarantees that complications caused by contamination due to atmospheric deposition or storage in museums can be avoided like what happens with other heavy metals (Thompson & Furness, 1989a; Furness, 1993). High levels of mercury in feathers do not necessarily imply pollution but may simply reflect natural dietary intakes or lacking the ability to excrete mercury. To clarify this situation geographical comparisons or time-series trends can be done (Furness, 1993; Becker *et al*., 2016).

4.1.4 Cory's Shearwaters' moult

Moult is a life cycle process that involves high energy cost and a substantial input of nutrients. It also compromises flight efficiency. Because of that it is rare for it to coincide with other energy demanding events, such as reproduction and migration (Newton, 2008; Alonso *et al.*, 2009).

Biogeochemical analysis of feathers is increasing and being used in tracing migratory movements and heavy metal monitoring studies. Due to that, the interest in spatiotemporal moult patterns is increasing. Knowing the spatiotemporal moult patterns is very useful because feathers' mercury concentrations reflect what the individuals were feeding on by the time and location where each feather was formed (Ramos *et al.*, 2009; Watanuki *et al.*, 2015).

In Cory's Shearwaters, moult starts within the primaries, in late August until late October (before migrating). After arriving from the wintering grounds most birds have completed their moult (Alonso *et al.*, 2009). A representation of Cory's Shearwaters feathers and moulting sequences is represented in figure 20.

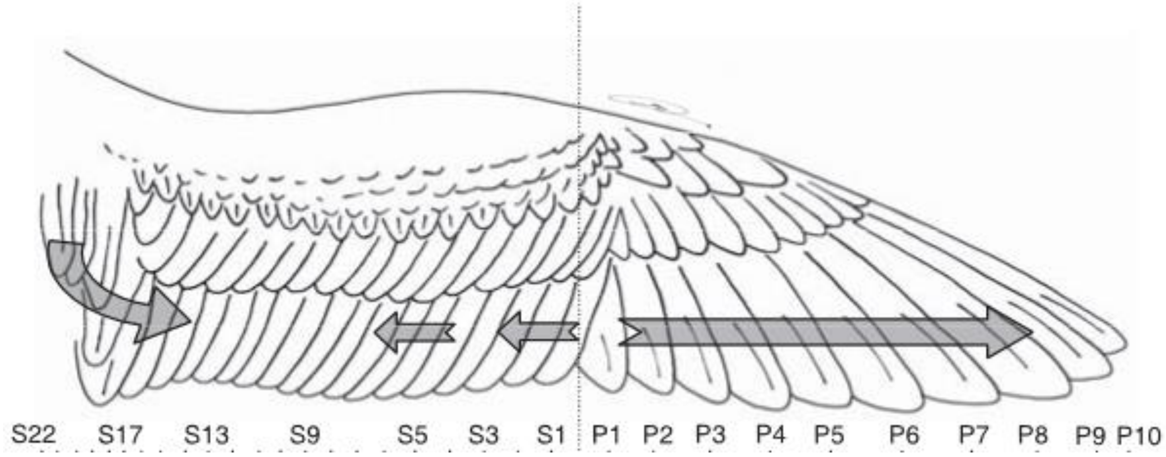


Figure 20-Cory's Shearwaters' secondaries moult patterns (Ramos *et al.*, 2009)

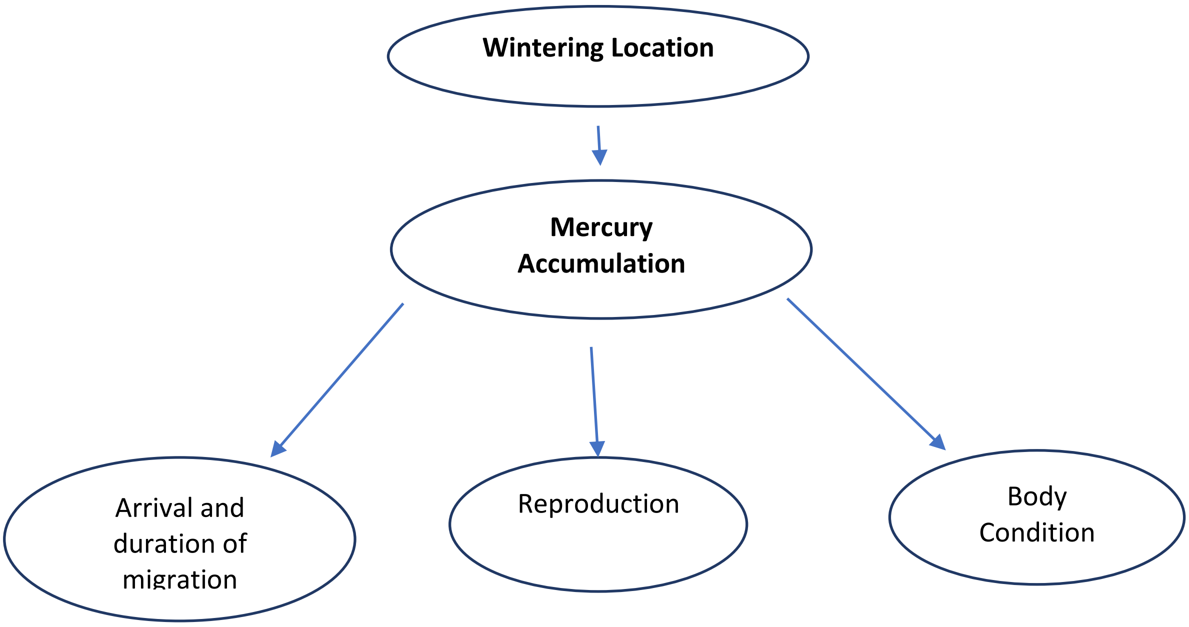
In recent studies it is shown that secondaries moult happens during the winter. For example, in a study done by Alonso *et al.* (2009), no individuals analysed in Selvagem Grande during September were moulting the secondaries, while later in March all the individuals had new secondary feathers, indicating that they had been moulted over the winter. In other study by Ramos *et al.* (2009) it was also referenced that secondary feathers moult in winter time, specifically secondary 8. Secondaries have a more complex moulting pattern, in comparison with primaries, for example. It has three different asynchronous foci. The first one to moult are the innermost secondaries (S21), secondly, the middle secondaries (S5) following by the outermost secondaries (S1) (Figure 21) (Ramos *et al.*, 2009).

4.1.5 Objectives

The present study adds new information on mercury exposure and its demographic consequences on the Cory's shearwaters breeding at Selvagem Grande.

In this chapter the objectives are to assess whether Cory's shearwater individuals experience different mercury exposure in the different wintering locations, describing body feather's mercury concentrations in these data set of individuals and, with that, provide information about geographical mercury distribution and contamination.

Another goal is to investigate if mercury accumulation in different wintering locations causes demographic consequences in terms of arrival dates at the colony, body condition, duration of migration and reproductive success.



4.2 Material and Methods

4.2.1 Sampling

While doing the daily nest checks between 2nd of March and 12 of April of 2017 in all the targeted nests in Selvagem Grande, all the individuals in which geolocators were collected were sampled. Two centimetres from the tip of secondary 8 (counting proximally) were collected using a scissors. Moult studies in this species suggest that this feather is very likely moulted in the wintering grounds. Only two centimetres from the tip were collected in order to minimize possible harmful effects on the individuals.

Since mercury is strongly bonded to keratin and not affected by rigorous treatments, feather samples were stored in plastic bags and no special treatment or wash was applied (Furness *et al.*, 1986).

4.2.2 Mercury Chemical Analysis

The chemical analysis was carried out at Aveiro University, in the Chemistry department.

Mercury amounts present in each individual's secondary 8 were assessed by the method established by Costley *et al*. (2000), using LECO AMA254 (Advanced Mercury Analyzer-Atomic Absorption Spectrometer) that is specifically designed to determine total mercury content in various solids and liquids without sample pre-treatment or sample pre-concentration. Small cuts from the sampled piece of feather were placed into a pre-cleaned combustion boat and inserted in a quartz combustion catalytic tube. The sample was firstly dried at 120 °C prior to combustion at 680-700 °C in an oxygen atmosphere. The mercury vapour released from the combustion is collected in a gold amalgamator and after a delay period heated at 900 °C. The mercury is then transported to a heated cuvette (120 °C) and quantified by atomic absorption spectroscopy using a silicon UV diode detector.

Samples were weighed prior to mercury quantification and the resulting mass of mercury was converted into mg of mercury per kg of feather.

The mercury recoveries were between 80 % and 100 % for the laboratory standards. Measurement quality and accuracy were certified by the certified reference material (CRM) SRM2976-freeze-dried mussel tissue (NRC, Canada) with a mercury concentration of 0.61 ± 0.04 mg/kg and the detection limit the analysis was 0.2 mg/kg (dry weight). Blanks were analysed at the beginning of each set of samples and mass of the CRM was adjusted to represent the same amount of mercury introduced in the AMA compared to the one in the feathers.

All samples were measured in duplicate and average values were used in all statistical tests. If the coefficient of variation on duplicate measures was >10%, then a third or more measure was carried out until coefficient of variation was <10% between repeated samples. The values with the largest deviation were excluded from calculation of the mean.

4.2.3 Data Analysis

A mean mercury concentration in mg/kg for each individual was obtained though feathers' samples chemical analysis.

Because feather mercury load did not follow a normal distribution (Shapiro-Wilk, $p<0.001$), non-parametric tests were done. In order to see if any difference in mercury concentrations was present between sexes a Mann-Whitney test was carried out. To assess possible differences in mercury concentration depending on wintering location a Kruskal-Wallis test was carried out with the groups being the different wintering locations and mercury the dependable variable. Kruskal-Wallis post hoc tests were used to see specific differences in mercury concentration between each wintering location. Relatively to mercury concentrations and reproduction success, a Mann-Whitney test was used to determine if mercury concentrations were different between individuals with or without an egg in the nest. A Spearman's rank-order correlation was done to determine if there was any significant relationship between mercury concentrations and body condition; between arrival dates and mercury concentrations; and between mercury concentrations and the duration of the return migration. The variables body condition and arrival dates are explained in chapter 3 – Data analysis. Duration of migration was defined as the time between the day an individual left wintering location and return to the nest in the colony. All these procedures were also repeated for both sexes separately and, when appropriate, for each wintering location.

All the statistical analysis was carried out using SPSS Statistics 24, with a significance level of 0.050.

4.3 Results

4.3.1 Mercury Levels

In a total of 142 individuals, mercury concentrations registered a mean of 5.8 ± 2.3 mg/kg. The minimum recorded was 1.7 mg/kg, and the maximum 19.7 mg/kg. After running a Mann Whitney test, it can be concluded that mercury concentrations were not significantly different between males and females ($U= 1.753$; $p=0.127$; $n=119$). The average mercury concentration for males was 5.8 ± 2.1 mg/kg and for females 5.4 ± 1.9 mg/kg.

Because there were no females wintering in the Canary current, if testing for differences in mercury levels between sexes without individuals from the Canary current the results were a mean mercury concentration of 6.1 ± 1.8 mg/kg for males and 5.2 ± 1.7 mg/kg for females, showing a significant difference between both ($U= 674.000$; $p=0.001$; n(males)=66 n(females)=34) (figure 21).

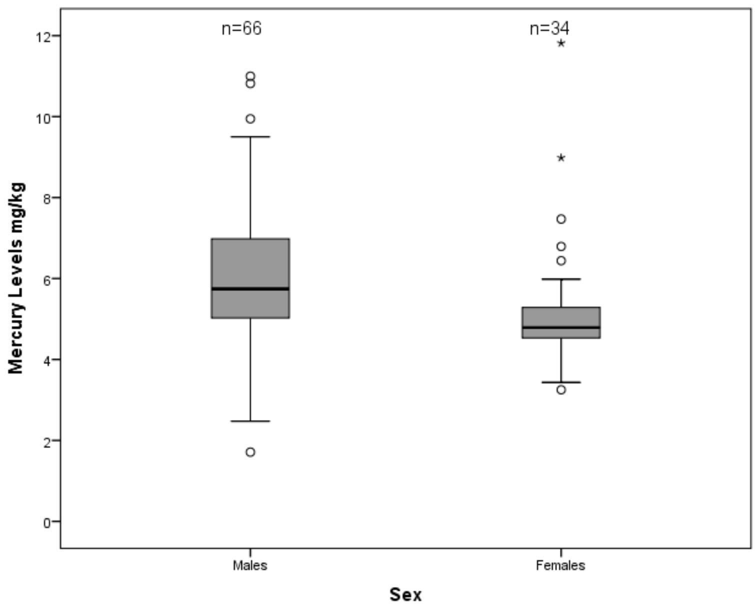


Figure 21-Differences in mercury levels (mg/kg) between sexes in Cory's shearwaters' individuals wintering in southern Africa locations

When testing the differences in mercury concentrations between sexes in each southern Africa wintering location the results revealed no significant differences between males and females from Benguela and from Agulhas **(**U= 223.000; p=0.148; n(males)=43 n(females)=14 and U= 18.000; p=0.282; n(males)=5; n(females)=11 respectively), but when analysing individuals wintering in "Cabo" (when it was not clear if individuals wintered in Benguela current or Agulhas current) there were significant differences in mercury concentrations between sexes (U= 28.000; p=0.014; n males= 19, n females=7).

4.3.2 Mercury concentrations relative to wintering locations

The mean mercury concentrations in each wintering site is shown in figure 22. Northeast Atlantic is the location with higher mercury mean (6.8 mg/kg), but the sample size is too small, so it cannot be taken as representative and it was not included in statistical tests, as well as South Atlantic and Brazilian Current. Besides Northwest Atlantic, Cabo is the location with higher mercury mean (6.1 mg/kg) and Canary Current with the lowest (4.1 mg/kg). Because the term "Cabo" was used when it was not clear if individuals wintered in Benguela current or Agulhas current, this location is not considered as an individual location.

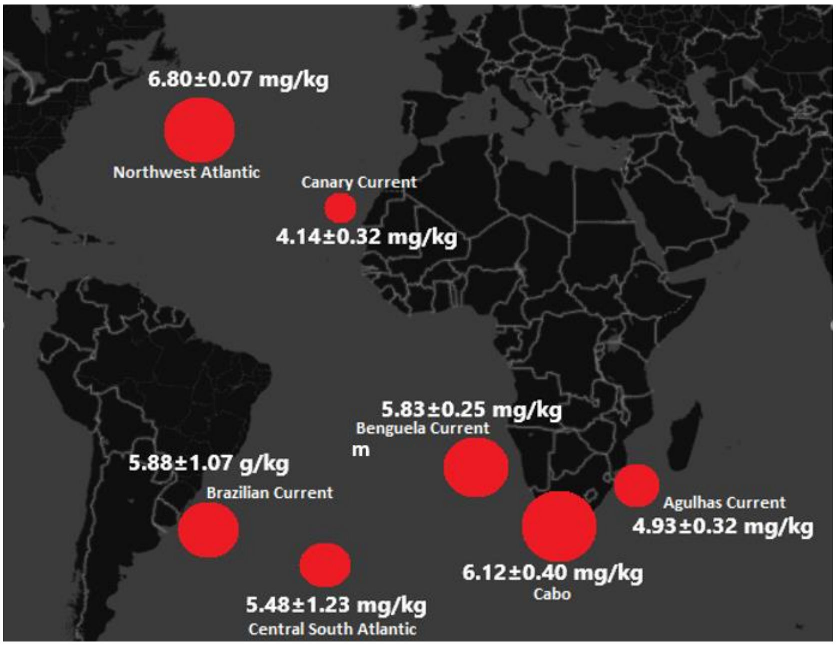


Figure 22--Mercury concentrations (mg/kg) found in Cory's shearwaters' individuals wintering in each location

Kruskal-Wallis test between wintering sites (except Northwest Atlantic, South Atlantic and Brazilian current) and mercury showed significant differences in mercury concentrations amongst the groups (H = 17.350, p = 0.001, n=127). The groups showing differences were Canary Current and Cabo (H = 38.336, p = 0.003, n=44) and Canary Current and Benguela (H = 35.867, p = 0.001, n=81) (figure 23). Canary current is the place with lower mercury concentrations.

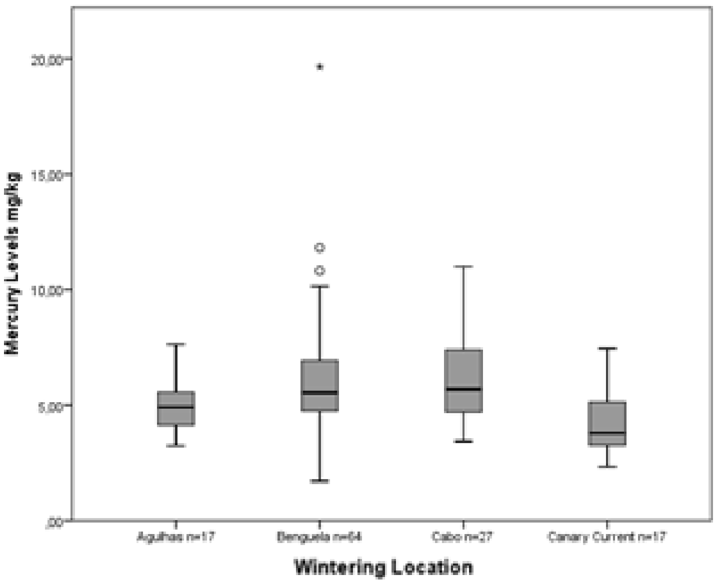


Figure 23-Differences in mercury concentrations (mg/kg) in Cory's shearwaters' individuals amongst wintering locations

If the analysis is made separately for each sex the results remain the same for males, showing significant differences between groups (H = 18.133, p < 0.001, n=88). Canary Current and Benguela (H = 23.181, p = 0.005, n=63) and Canary Current and Cabo (H = 33.585, p < 0.001, n=38) being significantly different (figure 24).

For females there was no significant difference between mercury concentrations in each wintering site (H = 0.967, *p* = 0.616, n=33).

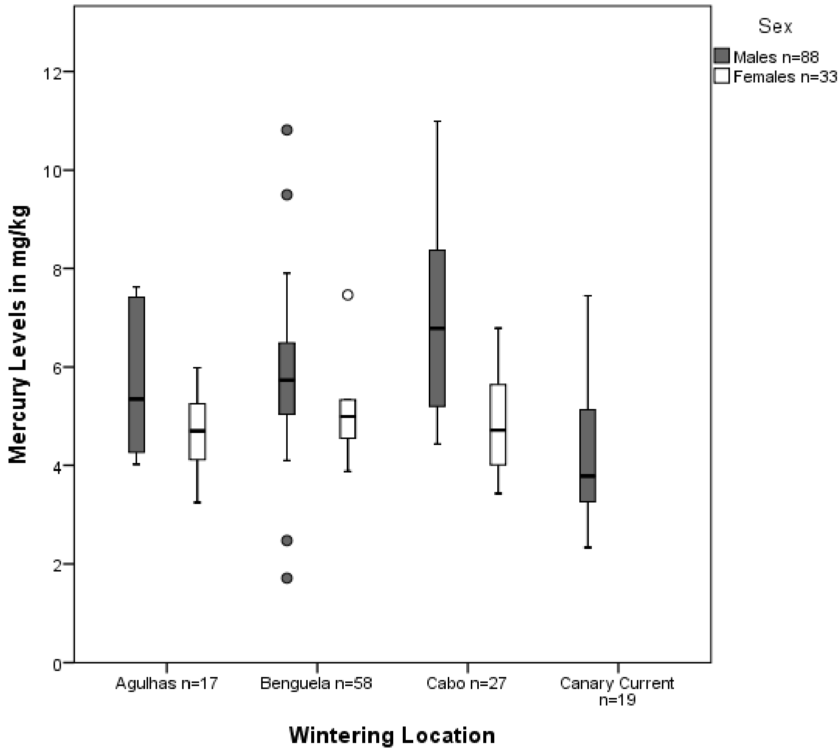


Figure 24-Differences in mercury concentrations (mg/kg) amongst wintering locations between sexes

4.3.3 Relationship between Mercury concentrations and Laying success

From the analysed data, it can be concluded that mercury concentration was not significant different between individuals who laid eggs and who did not (U= 2.287; p=0.224; n=146) (figure 25). There are also no differences testing each sex separately (males U= 1 107.0; p=0.225; n=109); (females U= 142.0; p=0.105; n=37).

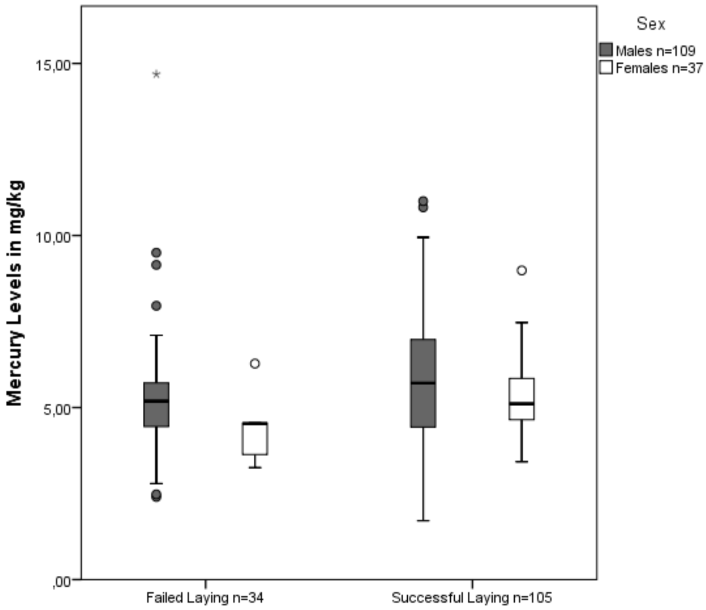


Figure 25--Differences in Cory's shearwaters' individuals laying success depending on mercury concentrations (mg/kg) for each sex

4.3.4 Relationship between Mercury concentrations and Body condition

There was no statistically significant correlation between mercury concentrations and body condition (r_s = 0.098, p = 0.323, n=107) (figure 26).

When testing only males there was no significant correlation (r_s = 0.191, p = 0.108, n=84), neither for females (r_s = 0.024, p = 0.880, n=43). The results are shown in figure 26. There was also no significant correlation between mercury concentrations and body condition amongst individuals wintering in Canary current (r_s = -0.099, p = 0.737, n=17) and southern Africa locations (r_s = 0.037, p = 0.730, n=90).

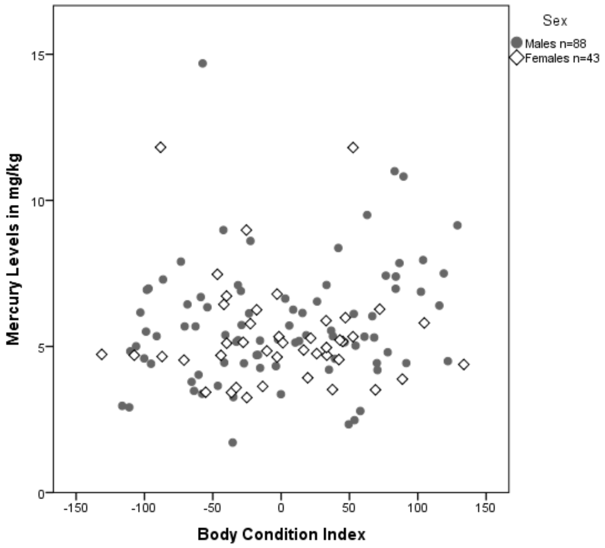


Figure 26-Relationship between Cory's shearwaters' body condition and mercury concentrations (mg/kg)

4.3.5 Relationship between Mercury concentrations and Arrival Dates

For males, the correlation between mercury concentrations and arrival dates at the colony was significant and positive (r_S= 0.397, n = 89, p < 0.001). For females the correlation was weaker and insignificant (r_S= 0.274, n = 42, p =0.080). The results are presented in figure 27. When testing only males from southern Africa there was also a significant positive correlation (r_S= 0.313, n = 68, p < 0.011) (figure 28) and between males from Canary current there was no significant correlation (r_S= 0.286, n = 17, p = 0.265).

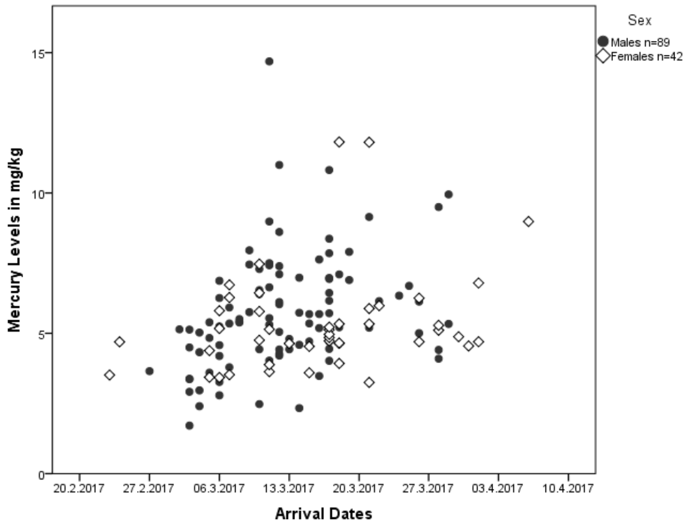


Figure 27-Relationship between Cory's shearwaters' arrival dates and mercury concentrations (mg/kg)

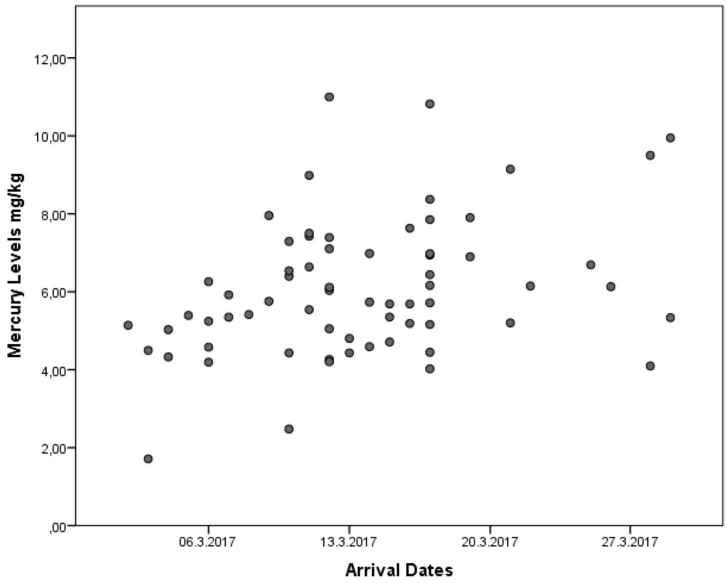


Figure 28-Relationship between arrival dates and mercury concentrations (mg/kg) in male Cory's shearwaters wintering in southern Africa locations

4.3.6 Relationship between Mercury levels and duration of return migration

The average migration duration between individuals was 27 ± 7 days.

Because individuals wintering in Canary current partially migrate they were not included in the tests.

There was no correlation between mercury concentrations and duration of migration for individuals wintering in Benguela (r_s= 0.130, n = 54, p =0.351); Agulhas (r_s= -0.184, n = 16, p =0.511) and "Cabo" (r_s= 0.006, n = 27, p =0.976), both males and females included (figure 29).

While analysing individually for each sex there was also no correlation between mercury concentrations and duration of migration (Males: Benguela (r_s= -0.277, n = 41, p =0.079); Agulhas (r_s= 0.205, n = 6, p =0.741) and "Cabo" (r_s= -0.216 n = 19, p =0.388); Females: Benguela (r_s= 0.323 n = 13, p =0.281); Agulhas (r_s= -0.169, n = 10, p =0.640) and "Cabo" (r_s= 0.229 n = 8, p =0.586)), although in males from Benguela this value was not far from statistically significant.

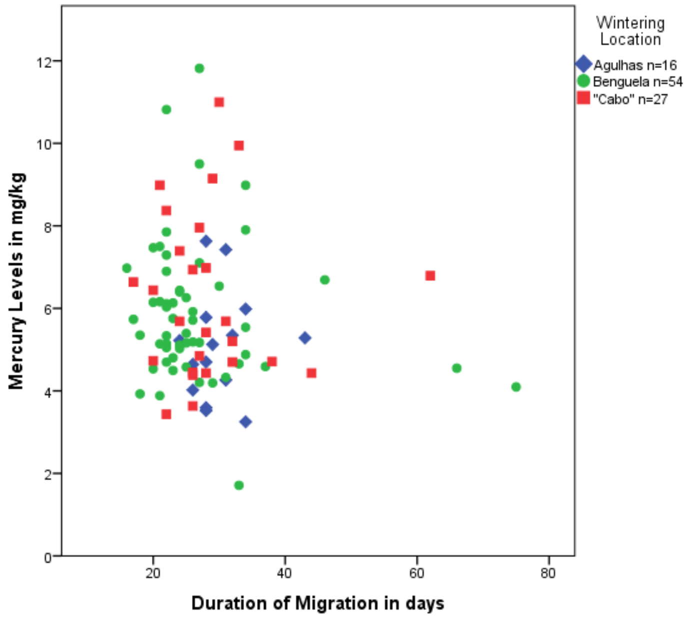


Figure 29-Relationship between Cory's shearwaters' mercury concentrations (mg/kg) in individuals wintering in each location and the duration of migration (days)

4.4 Discussion

The mean mercury concentration deposited in winter growth feathers of adult Cory's Shearwaters was 5.8 ± 2.3 mg/kg, with differences between males and females. Males wintering in the Canary current registered the lowest mercury burden when comparing with birds spending the winter in southern Africa locations. Mercury concentrations did not seem to affect laying success, body condition and duration of migration amongst individuals. However, there was a positive correlation between arrival dates from southern Africa males and mercury concentrations, in which individuals arriving later had higher mercury concentrations. The wintering location with lower mercury concentrations was Canary current with 4.14±0.32 mg/kg.

A study by Ramos et al. (2009) carried out in 2000 with Cory's shearwaters individuals wintering in the Canaries recorded mercury concentrations in a range between 3.4-3.6 mg/kg also using the eight secondary. When comparing these values with the ones obtained in the present work, it suggests that there was an increase in the mercury burden in the Canary Current region.
Most of the times the chosen feathers to do this kind of analysis are body feathers, which are often believed to be more accurately representative of body's mercury burden (Furness et al., 1986), instead of flight feathers. Because of that there is not many literature with values that can

be used as a reliable comparison for the ones from this work. When comparing these values with ones from previous studies in North and Mid Atlantic using body feathers there were some differences to be noticed. In a Monteiro et al. study (1995), carried out between 1990 and 1992 the mean mercury concentration in Cory's shearwaters' body feathers from the Azorean archipelago was 6.3±0.33 mg/kg. Between 1993 and 1995, Monteiro et al. (1998) recorded a mean mercury concentration of 5.4±0.1 mg/kg also in individuals from the Azorean Archipelago. In another study also by Monteiro et al. (1999), within the same period of 2 years, the obtained mercury concentrations in individuals from Selvagem's Archipelago was 5.2 ±1.6 mg/kg. These numbers are shown in table 2 as well as others presented by Monteiro & Furness (1997) for the same regions.

Table 1-Mercury levels recorded in feathers from previous literature in Cory's shearwaters from North and Mid Atlantic colonies (adapted from Monteiro *et al.*, 1997). BF=body feathers; S8=secondary eight

	1885-1900	1900-1931	1950-1970	1990-1992	1993	1993-1995	2000-2001	2016-2017
Calonectris borealis	1.8±0.2 mg/kg	2.9±0.1 mg/kg	3.9±0.5 mg/kg	6.3±0.33 mg/kg Monteiro *et al.*, 1995	5.4±0.1 mg/kg	5.2±1.6 mg/kg Monteiro *et al.*, 1999	3.4-3.6 mg/kg Ramos *et al.*, 2009	4.1±0.3 mg/kg
Feather	BF	BF	BF	BF	BF	BF	S8	S8

Observing these values from Northeast and Mid-Atlantic it is possible to see an increase in mercury levels since 1885 until the beginning of the 1990's, where they seem to have reached a maximum. This is explained and referred in previous works, for example by Monteiro & Furness (1997) in which these mercury trends are consistent with the global increases in atmospheric and sea surface mercury since pre-industrial times caused by anthropogenic inputs in North Atlantic (Slemr & Langer, 1992; Burgess et al., 2013; Pollet et al., 2017). From there, the levels of mercury in this part of the Atlantic appear to have decreased since numbers went from 6.3 ± 0.3 mg/kg in 1990-1992 to 4.1 ± 0.3 mg/kg in 2016-2017. This decrease could be explained by the implementation of environmental measures in developed countries surrounding this ocean (Driscoll et al., 2013; Weigelt et al., 2015), which led to a slow decrease in mercury emissions, despite the global trend still towards an increase of mercury emissions. However, these assumptions need to be carefully thought since the mercury concentrations obtained in this study are referent to a secondary feather and the ones from the studies in comparison refer to body feathers, which can have differences in terms of mercury intakes.

When comparing it with the study carried out by Ramos et al. (2009) where the eight secondary was used, mercury levels recorded where higher in the present work, although the difference was low, suggesting a slight increase since the year 2000, which could indicate that although mercury emissions are decreasing in countries surrounding North and Central Atlantic, the global trend still walks towards an increase in mercury emissions. This can be explained by the recent industrial development in developing countries that are now thriving and emitting mercury that spreads globally across the atmosphere. Another explanation to an increase in mercury levels that does not require an increase in mercury emissions is the one related to atmosphere composition. Changes in the general chemistry of the atmosphere, like a high quantity of oxidants and an increase in acidification, are known to somehow enhance mercury deposition. This means that global and local emissions can remain stable and mercury levels recorded in living organisms can still become higher due to an increase in oxidants and acidification of the atmosphere (Monteiro & Furness, 1995).

The mean levels of mercury recorded in the present work were slightly higher than the level sometimes associated with failed reproduction in marine and terrestrial birds (5 mg/kg) (Burger & Gochfeld, 1997; Watanuki et al., 2015), being 5 mg/kg commonly used to assess toxicity threshold in feathers (Watanuki et al., 2015; Bustamante et al., 2016). However, the mercury concentration threshold in feathers is still a target of various studies and depends of various factors like dietary composition, moult duration, phylogeny and ability to demethylate mercury in the liver (Kim et al., 1996; Bond et al., 2015). In previous literature, some different thresholds have been used. Burger & Gochfeld (1997) considered that mercury concentrations in feathers between 5 and 40 mg/kg could cause adverse effects in marine and terrestrial birds. For piscivorous loons, a threshold level in a range of 10 to 15 mg/kg is known to cause adverse effects (Evers et al., 2014), as well as for bald eagles (Haliaeetus leucocephalus) (Cristol et al., 2012; Bond et al., 2015). Bond et al., considered a threshold level of 20 mg/kg of mercury for ivory gulls (Pagophila eburnea). Despite these thresholds, there are records of over 50 mg/kg of mercury in feathers from some procellariiforms from North and South Atlantic remote islands (Muirhead & Furness, 1988; Monteiro & Furness, 1995). There are even mercury levels from one wandering albatross individual of 75 mg/kg in the southern Indian ocean (Thompson et al., 1993). In these examples, mercury levels were much higher than the levels associated with failed reproduction and toxicity threshold and there were no apparent negative consequences for the individuals. There is a great variation in mercury concentrations present in feathers and it varies within a large range amongst different species (Thompson, 1990; Monteiro & Furness, 1995) which can lead to believe that there is still a lack of information about toxicity levels of this heavy metal in seabirds. However, in the present work the mean mercury levels only stayed above the threshold of 5 mg/kg, staying under the ones previously mentioned.

When looking at mercury levels for both sexes separately, there were differences noticed between males and females from southern Africa locations. The average mercury concentration in southern Africa locations for males was 6.1 ± 1.8 mg/kg mg/kg and for females 5.2 ± 1.7 mg/kg. This variation could be explained due to the fact that females can excrete up to 20% of their mercury burden through egg laying (Lewis et al., 1993; Furness & Camphuysen, 1997) and also due to sexual segregation in foraging areas and diet (Ramos et al., 2009; Bustamante et al., 2016). Cory's shearwaters males and females have different dietary regimes (Alonso et al., 2014), and this could be because of males' tendency to be larger than females, so they can be more apt to ingest larger preys, with potentially higher mercury burdens (Ramos et al., 2009: Alonso et al., 2014). Dietary differences can cause variation between sexes, but it also makes it difficult to compare mercury levels between males and females and relate females' values with levels excreted in eggs. The mercury levels did not vary between males and females wintering in Benguela current and wintering in Agulhas current. However, there were significant differences between individuals wintering in "Cabo", which designation was used when it was not clear if individuals wintered in Agulhas current or in Benguela current. This suggests that the differences in mercury concentrations in males and females wintering in southern Africa locations can be due to sex-biased diets and/or different niche use between sexes in these different wintering locations.

Individuals wintering in Benguela had higher mercury concentrations than the ones in Canary current, with 5,8±0,3 and 4,1±0,3 mg/kg respectively, and there were significant differences between Canary current individuals' mercury levels and the ones in southern Africa locations. This is contrary to what is said in other studies (Slemr & Langer, 1992; Monteiro et al., 1995). These differences can be due to different dietary intakes amongst individuals in different wintering locations, according to the availability of preys (Alonso et al., 2014). However, these results are in accordance with some recent studies saying that mercury emissions in North Atlantic are known to be decreasing in the last years due to environmental measures carried out by the countries that in the past were the major responsible for mercury contaminations. According to Soerensen et al. (2012) mercury concentrations in the air from the sub-tropical zone of the Atlantic Ocean are predicted to be decreasing between 0.025 and 0.035 ng m^{-3} yr^{-1} as well as mercury concentrations in seawater (Driscoll et al., 2013), presumably because of the decreasing emissions of North America and Europe (Driscoll et al., 2013; Weigelt et al., 2015). This study by Soerensen et al. (2012) also showed an 80% decline in mercury concentrations since 1980 to 2012 in the referred area. It is not likely that mercury levels registered in the end of the last century still influence actual levels, since mercury lifetime is about one to two years in seawater and at the air layer in contact with water's surface (Bank, 2012) and the hemispheric air mixing has a duration of approximately three months (Warneck, 1988). Although North Atlantic mercury concentrations seem to be decreasing, or at least increasing at smaller rates, the overall trend for all the oceans goes in the opposite direction, which can be seen in the overall higher rates in mercury concentrations in the studied individuals when comparing with older literature. Recently developing countries in South and East Asia are responsible for an increase in overall Northern Hemisphere atmospheric mercury levels, which are then integrated in the ocean affecting also the southern hemisphere (Driscoll et al., 2013; Weigelt et al., 2015). This could explain the lower mercury levels obtained for individuals in Canary current comparing to southern Africa locations, since Canary current is situated in North Atlantic and influenced more by water currents coming from further north were mercury concentrations are thought to be decreasing and nowadays achieving lower levels than in South Atlantic (Pacyna et al., 2016; Martin et al., 2017).

In southern Africa locations, especially western ones, mercury concentrations trends are not well understood compared to the North Atlantic ones (Read et al., 2017). However, it is believed that there is an increasing trend in mercury levels. An example of that is a report made by Martin et al. (2017) showing an increase in atmospheric mercury concentrations in Cape Point (South Africa) between 2007 and 2015, and this could explain the higher mercury levels recorded in individuals wintering in southern Africa locations.

A study carried out by Pacyna et al. (2016), about global anthropogenic mercury emissions, shows global trends about mercury emissions. It indeed confirms that mercury emissions decreased in Europe and increased in Africa since 1980. A continuous decrease of mercury emissions in Europe can be explained by installation of emission control equipment in Western Europe, particularly highly efficient electrostatic precipitators at the beginning of the 1980s and flue gas desulphurization (FGD) technologies towards the end of the 1980s, and a change from coal to oil/gas for energy production in several countries. The African emissions have increased mainly due to the increase of population in the continent, and resulting enhanced demand for energy, as well as due to increasing gold production using mercury recovery methods. Another study done by Wilson et al. (2006) mapped the global mercury emissions (figure 30), showing higher mercury emissions in southern Africa and lower in the Canary current zone. Mercury concentrations found in Cory's Shearwaters from this study seem to match these trends (lower mercury levels in Canary current and higher ones in southern Africa locations).

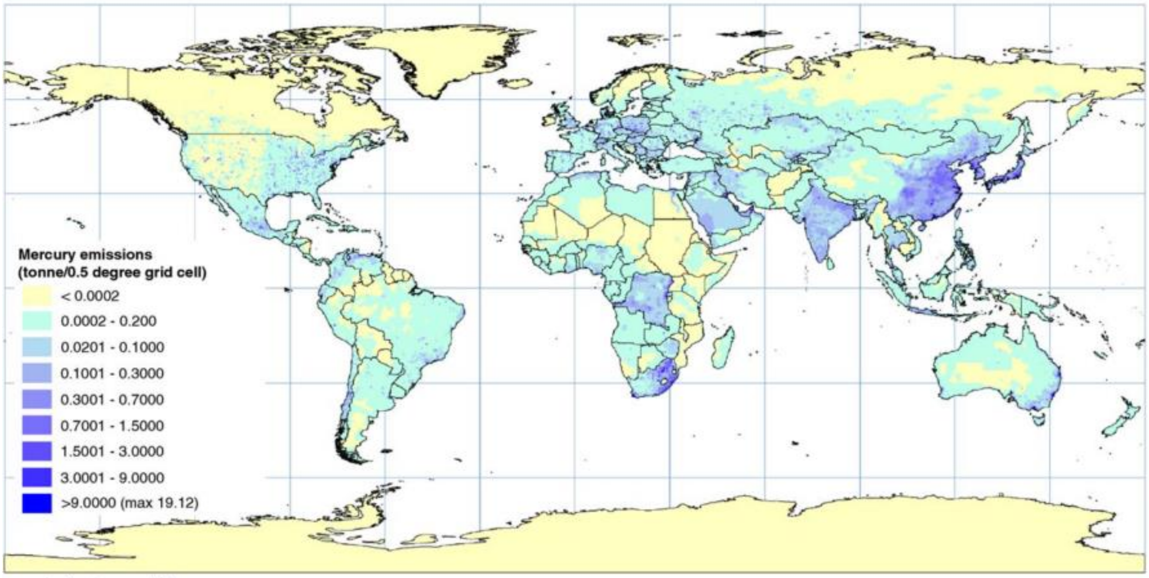


Figure 30-Global anthropogenic mercury emissions in 2000 (Wilson *et al.*, 2006)

In this study there was no influence of mercury concentrations in laying or hatching success. This agrees with previous studies for other bird species in which reproductive success was not affected by mercury concentrations (Thompson et al., 1991; Mitro et al.,2008; Bustamante et al., 2016). For example, Thompson et al. (1991 and 1992) reported a mean mercury levels of 7 ± 5.1 mg/kg in adult Great Skuas (Catharacta skua) and a mean of 7.21 ± 1.18 mg/kg in North Atlantic Gannets (Morus bassanus) without any apparent effects in reproductive success. Although the concentrations associated with failed reproduction are often exceeded in seabirds, this does not seem to affect individuals (Bond et al., 2015). Naturally high exposure of seabirds to mercury in the food chain may have led to the evolution of detoxification mechanisms (Muirhead & Furness 1988). This may be the case in the studied individuals, suggesting a level of adaptation to higher mercury burdens which could be toxic for other organisms, including human beings. Although these individuals are well adapted to higher mercury concentrations they could indicate high mercury levels in food chain, especially in fish, that can be consumed by humans and became harmful.

There was no apparent relationship between mercury levels and body condition. This does not match with a previous study by Wayland et al., (2002) in another seabird's species, where it was recorded an inverse correlation between body condition and mercury concentrations. This is another point towards a lack of apparent influence of mercury load in the individuals, since one of the possible adverse effects of mercury is reduced food intake leading to weight loss and progressive weakness in wings and legs (Ochoa-acuña & Gross, 2002). However, there are still doubts if this applies to Procellarids (Ochoa-acuña & Gross, 2002).

Another factor that is known to influence mercury burden is feeding specialisation, causing inter-species and intra-species variation (Monteiro et al., 1998). This is connected to epipelagic or mesopelagic habits. Mercury accumulation is far greatest in mesopelagic preys than in epipelagic ones (due to the low levels of oxygen increasing methylmercury formation), because of that, individuals with epipelagic feeding habits are expected to have lower mercury levels than the ones with mesopelagic feeding habits (Mason & Fitzgerald, 1993). It would be expected that individuals feeding on coastal shelves had lower mercury concentrations than the ones in deep waters since increased concentrations of methylated mercury compounds are produced in low oxygen waters (Monteiro et al., 1996). Cory's shearwaters take mainly epipelagic prey, but with some mesopelagic prey also present in the diet (Monteiro et al., 1998). Both individuals from Canary current (lower mercury levels) and Benguela current (higher levels) wintered mostly in continental shelves, indicating that the main feeding was done in epipelagic environments, agreeing with previous studies like the one done by Monteiro et al. (1998). If mercury concentrations were only related to feeding habits it would be expected that male individuals from both of these regions had similar mercury burdens, on average, which was not the case. Since the diet of these seabirds is composed mostly by epipelagic prey, this species can be thought of as monitors of epipelagic waters.

The obtained results suggest that latter arrival dates were associated with higher levels of mercury in males from southern Africa. Despite the lack of studies connecting mercury levels and arrival at breeding grounds, the results in the present work did not match with a study carried out by Provencher et al. (2016) where a relationship between mercury and arrival date was not found, although the region was not the same as in this study. Higher mercury burdens could influence flight ability and because of that causing birds to arrive latter. Another adverse effect caused by higher mercury concentrations is movement incoordination and difficulty in flying (Ochoa-acuña

& Gross, 2002), so, it should be expected that individuals with higher mercury levels) faced more difficulties in migration flight and, as a consequence, taking more time migrating. Besides motor problems, mercury can also affect the endocrine system or other mechanisms that can have an important role regulating the annual life cycle (Monteiro & Furness, 1995). However, there was no relationship between migration duration and mercury levels, indicating once more a possible degree of adaptation.

This study provided results that can reinforce the fact that seabirds can effectively be used to track long-term changes in mercury contamination on a large scale. The obtained mercury levels agree with predictions for this contaminant emissions over the years. Seabirds can act as monitors of mercury in marine environment, and although mercury did not seem to have major effects in these individuals it seemed to affect arrival time at the colony and it seemed to somehow vary between males and females, showing possible "carry-over" effects. However, these did not seem to have any negative effect in reproduction success. Also, the monitorization of mercury levels can provide useful information to assess possible implications of the human exploitation of the epipelagic environment and to the implementation of measures to reduce mercury emissions, like for example the ones implemented in European industries by the end of the last century.

CHAPTER 5 - GENERAL CONCLUSION

This study provides information about migratory behaviour of Cory's Shearwaters and also new information about how their migratory behaviour affects traits like body condition, arrival dates, reproduction and mercury accumulation. It benefits from the long term research that has been carried out in Selvagem Grande, enabling to relate factors like sex with the study variables and improve GLS retrieve rates.

These results showed the importance of carry over effects from different wintering grounds, which in this case may be causing a decrease in reproduction success due to lower body condition or a sex biased population. At first sight, the most probable factor that could decrease reproduction success would be mercury levels present in the individuals, but this was not verified. On the contrary, birds in the Canary current, with the lower mercury concentrations, were the ones with higher percentage of failed laying. This can indicate a degree of adaptation. However, higher mercury levels were associated with a latter arrival at the colony. Although it did not seem to affect reproduction, it suggests that these mercury concentrations can somehow affect the annual cycle and endocrinal system of individuals.

The present work only provides information about mercury levels in locations where tracked birds wintered, but it demonstrates the utility of this technique for monitoring the spatial pattern of mercury pollution in regions difficult to study, requiring a challenging logistic. In this case the results matched the predicted trends (Wilson *et al.,* 2006) and they were useful to reinforce information about the recent global mercury distribution, complementing previous studies. It also provides information about mercury concentrations in food chains present in the Atlantic Ocean surrounding southern Africa, where this kind of information is scarce.

All the collected data acts as a contribute and can be applied to identify and manage marine protected areas, since it provides information about this species distribution and influences of its distribution in its life choices. Using this species as a biomonitor for environmental pollution also provides important insights about levels of contaminants, in this case mercury, present in wintering areas, where data is scarce. Despite that, in future studies it would be important to have in mind that studies using feathers to assess the winter contaminant levels in birds with long breeding periods like Cory's shearwaters should consider the carry-over effects of mercury load that can occur between seasons (Ramos *et al*., 2009) and because of that, with more time, would be relevant to measure mercury concentrations in feathers representing the mercury intake in the breeding season. Also, including isotopes studies and investigating the relationships among stable isotope measurements and contaminants would help to elucidate the differential exposure of birds to contaminants in relation to their ecology.

Overall, it was demonstrated the importance of comprehending the linkage between wintering locations' conditions and demographic processes in populations.

CHAPTER 6 – REFERENCE LIST

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